Queen Mary University of London

The indirect impacts of ecosystem engineering by invasive crayfish

Guillermo Eduardo Willis-Jones Tambo

Submitted in partial fulfillment of the requirements of the Degree of Doctor of Philosophy

I, Guillermo Eduardo Willis-Jones Tambo, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without the prior written consent of the author.

Signature:

Date:

Details of collaboration and publications:

I have co authored a book chapter on crayfish ecology:

Willis-Jones E., Jackson M.C. & Grey J. (2016) Environmental Drivers for Population Success: Population Biology, Population and Community Dynamics. In: *Biology and Ecology of Crayfish*. (Eds M. Longshaw & P. Stebbing), pp. 251–286. CRC Press.

Abstract

Bioturbation by invasive crayfish can significantly alter sediment properties and its transport in invaded water bodies; however, the indirect impacts of this on ecosystem functioning are poorly understood. In this thesis I present data from mesocosm and field manipulation experiments used to assess the effect of bioturbation by three widely distributed invasive crayfish species (*Procambarus clarkii, Pacifastacus leniusculus* and *Astacus leptodactylus*) on a variety of ecosystem properties across seasons. In the mesocosm experiments, *P. clarkii* caused significantly more bioturbation than the other species, although increased bioturbation by all species in the spring and/or summer was associated with: reduced dissolved oxygen concentrations in near-surface water, indicating a large increase in oxygen demand by the water column; increased methane oxidation potential within the water (MOP_{wat}), indicating the re-suspension of methane oxidising bacteria (MOB) along with the sediment; and a shift in zooplankton community structure towards dominance by large cladoceran species. Stable isotope analysis of the zooplankton showed a strong relationship between $\delta^{13}C$ and MOP_{wat}, suggesting that bioturbation increases MOB consumption. Given the importance of zooplankton as a trophic link to the higher food web, crayfish bioturbation may increase the importance of methane derived (chemosynthetic) carbon in invaded ecosystems. Temperature was identified as the key driver of seasonal variations in crayfish bioturbation intensity through laboratory mesocosm experiments, enabling estimation of the full annual pattern of bioturbation intensity for each species. The optimal temperature for *P. clarkii* was much higher than for the other species meaning that its bioturbation impacts exhibited large seasonal fluctuations whilst *P. leniusculus* and *A. leptodactylus* maintained a lower but more consistent level. Field manipulation experiments of enclosed sections of Chalgrove Brook, Oxfordshire, found significant bioturbation activity by *P. leniusculus* in early autumn; however, the increase in turbidity was too small to detect other effects observed in the mesocosm experiments.

Acknowledgements

Firstly I would like to thank my supervisors Professors Jon Grey and Mark Trimmer for all their help and support over the course of my PhD. Their advice and enthusiasm have been indispensible. I also wish to thank the other members of the Grey and Trimmer labs for their advice and help. Particular thanks go to Felicity Shelley and Paul Fletcher for their invaluable practical and technical support.

I am also extremely grateful to my friends and family outside of QMUL; particularly my parents and sister who have always been ready with interest, support and encouragement. Importantly, I must thank Clare, your support and patience has been invaluable; I could not have done it without you!

I would like to thank Bob Campbell for giving me access to his property and allowing me to conduct experiments on his section of the Chalgrove Brook. In addition, I would like to thank Adrian Brooker at the City of London Corporation for giving me access to the Hampstead heath ponds to catch red swamp and Turkish crayfish and Jamie Godson of Lee Valley Park Authority for providing me with virile crayfish.

This work was supported by a Natural Environment Research Council CASE studentship with the Environment Agency.

Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1 Ecosystem Engineering and Bioturbation

Ecosystem engineers create, modify or maintain habitats by causing physical state changes in biotic and abiotic materials that, directly or indirectly, modulate the availability of resources to other species (Jones *et al.*, 1994). In so doing they can dramatically alter the biodiversity and functionality of their ecosystem. Physical resources that may be affected by ecosystem engineers are varied and include living space, heat, water, sediment and light. For example: a tree in a forest provides living space on its branches leaves and roots; casts shade and reduces the impacts of wind and rain (Callaway & Walker, 1997); aerates and binds the soil, reducing the effect of extreme weather events such as hurricanes (Tisdall & Oades, 1982; Basnet *et al.*, 1992); and creates new habitat such as tip-ups when it dies (Peterson *et al.*, 1990).

Many examples of ecosystem engineers can be found in the literature (Jones *et al.*, 1994, 1997; Crooks, 2002) since they have received increasing attention in the last two decades, but of particular interest are those species that are also invasive alien species (IAS) since they have the potential to dramatically alter their recipient ecosystem through cascading effects on other biota (Crooks, 2002). Freshwaters are considered to be particularly at risk from IAS since their high degree of human intervention renders them less resilient to change (Dudgeon *et al.*, 2006) and with rates of invasion showing no sign of slowing, despite national and international legislation (Jackson & Grey, 2013), the size of this problem is only set to increase. Therefore, effective strategies to conserve biodiversity and ecosystem services in the presence of IAS are required; however, this necessitates extensive knowledge of the effects of each new species, which can be very difficult to acquire due to the complexity of ecological systems and the large number of species involved. Consequently, much research on this topic has focused on IAS that are ecosystem engineers because they can effect large changes across entire ecosystems.

In their native habitats, ecosystem engineers often increase habitat heterogeneity, thereby increasing biodiversity and providing a greater variety of ecosystem services. Conversely, when introduced outside their native range, ecosystem engineers can produce conditions that native species are poorly adapted for, thereby having an adverse impact. For example, in their native range the North American and European beavers, *Castor canadensis* and *Castor fiber*, create large open patches of wetland in otherwise relatively continuous forest, thereby increasing fish and plant species richness at the local and landscape scale (Wright *et al.*, 2002; Smith & Mather,

2013) and reducing the risk of downstream flooding and low flows by reducing peak flow rates and increasing catchment storage capacity, respectively (Westbrook *et al.*, 2013; Puttock *et al.*, 2017). In contrast, the introduction of beavers in Chile resulted in the reduction of macroinvertebrate diversity due to loss of benthic microhabitats (Anderson & Rosemond, 2007). It is therefore clear that understanding how invasive ecosystem engineers in particular impact upon their recipient ecosystems is crucial to combating the degradation of those systems.

Invasive ecosystem engineers affect their recipient ecosystems in a large number of ways, but in aquatic environments one of the most important engineering processes is bioturbation, which is the biological reworking of sediments that is produced by any interaction between an animal and the sediment (Meysman *et al.*, 2006). This sediment reworking can affect ecosystem properties and processes in a variety of ways. If the animal producing the sediment disturbance is relatively large bodied then large amounts of sediment resuspension are likely to occur, which can increase water column turbidity, reduce light penetration and clog pore spaces in gravels and animal gills upon resettlement (Wood, 1997; Bilotta & Brazier, 2008; Harvey *et al.*, 2011). The resuspension of sediment through either biological or physical means alters sediment transport, since suspended sediment is carried by currents to other locations where it is deposited, thereby altering the physical structure of the sediment surface (Harvey *et al.*, 2011; Rice *et al.*, 2016). Sediment resuspension can promote aerobic decomposition of suspended organic matter (Bastviken *et al.*, 2004) and potentially the release of buried contaminants and nutrients which can cause toxic pollution (Feng *et al.*, 2008) and eutrophication (Angeler *et al.*, 2001; Rodríguez *et al.*, 2003). Furthermore, recruitment of toxic bloom forming cyanobacteria from the re-suspended sediment (Yamamoto, 2010) may further compound these effects. The subsurface structure can also be significantly altered by burrowing, with extensive tunnel networks in the bed and banks causing increased rates of erosion and bank collapse, thereby further contributing to sediment resuspension, transport and physical restructuring (Davis, 1993; Barbaresi *et al.*, 2004b; Johnson *et al.*, 2010; Harvey *et al.*, 2011).

The sediment of aquatic ecosystems acts as a reservoir of nutrients, organic compounds and a wide variety of solutes with concentrations in pond sediment typically being 2–4 orders of magnitude higher than in the water above (Boyd, 1995). Chemical exchange is constantly occurring across the sediment-water boundary but the physical structure of the sediment is extremely important in determining the biogeochemical processes that occur in, on and around it (Nogaro *et al.*, 2008). Bioturbation loosens the physical structure of the sediment and therefore facilitates the movement of solutes, thereby increasing the diffusive and advective

transport of solutes across the water-sediment boundary. For example, bioturbation by both common carp (*Cyprinus carpio*) and chironomid larvae has been found to reduce dissolved toxic metal concentrations through increased sequestration in the sediment (Ritvo *et al.*, 2004; Haas *et al.*, 2005). Conversely, benthic fish bioturbation can significantly increase nutrient flux from the sediment to the water both directly through increased advection and indirectly through consumption of buried organic matter followed by excretion (Schaus & Vanni, 2000; Ritvo *et al.*, 2004).

Perhaps the most ecologically important biogeochemical feature of sediments is oxygen concentration. In undisturbed sediments, oxygen can only penetrate the top few millimetres of sediment, resulting in a shallow oxic surface layer, underlain by a deep anoxic layer. In bioturbated sediments, oxygen penetration can increase because burrows and disturbance increase advection through a process known as bioirrigation (Kajan & Frenzel, 1999; Baranov *et al.*, 2016) and so oxic processes in the sediment can be promoted. For example, chironomid larvae burrows increase methane oxidation in the surface sediment (Kajan & Frenzel, 1999) whilst carp and tubificid worm bioturbation promotes aerobic decomposition of organic matter (Mermillod-Blondin *et al.*, 2004; Ritvo *et al.*, 2004). Consequently, the dynamics of ecologically and environmentally important substances, such as nutrients and methane can be altered by sediment bioturbation.

1.2 Crayfish as ecosystem engineers

In freshwater systems, many crayfish species are considered to be problematic invaders when introduced outside of their natural range (Strayer, 2010). In large part this is due to their highly omnivorous feeding habits which can dramatically alter the community structure of their recipient ecosystems. For example, the abundance of the American signal crayfish, *Pacifastacus leniusculus*, in Swedish ponds has been correlated with a decline in macrophyte and invertebrate biomass, with some taxa, such as Gastropda and Odonata, being more heavily affected than others, such as Chironomidae, thereby completely altering community structure (Nystrom *et al.*, 1996). Similar effects have been observed for other species such as *Orconectes rusticus* and *Procambarus clarkii* (Lodge *et al.*, 1994; Gherardi & Acquistapace, 2007). In addition, several crayfish species originating in North America carry a disease called crayfish plague, *Aphanomyces astaci*, which in combination with increased competition has the potential to cause the local extinction of native crayfish species (Cioni & Gherardi, 2004; Gil-Sánchez & Alba-Tercedor, 2006; Holdich *et al.*, 2009).

Despite their widespread impact, direct predation, competition and disease transmission are not true ecosystem engineering processes since they do not create or modify habitat (Jones *et al.*, 1994), however, crayfish also undertake true ecosystem engineering through macrophyte removal, excretion and bioturbation. Consumptive or incidental destruction of macrophytes and filamentous algae by crayfish alters relative habitat availability, thereby indirectly impacting the population success of species that utilize the destroyed or newly created habitat. For example, grazing by *Orconectes propinquus* can practically eliminate filamentous algae in deep-water environments, resulting in indirect facilitation of epilithic diatoms and sessile, grazing insects that consume these diatoms (Creed Jnr, 1994). Removal of macrophytes can also make water bodies more susceptible to a shift from a clear-water to a turbid phytoplankton dominated state, an effect that has been observed after *P. clarkii* invasion (Rodríguez *et al.*, 2003). Crayfish excretion can also have an ecosystem engineering effect since crayfish consume large quantities of particulate organic matter, increasing detritus processing rates and thus nutrient release upon excretion (Creed & Reed, 2004; Zhang *et al.*, 2004; Evans-White & Lamberti, 2005).

The primary physical ecosystem engineering impact of crayfish is bioturbation. Crayfish are very large benthic invertebrates that can occur at relatively high densities which means that any activity they undertake such as burrowing, foraging or fighting can significantly alter the physical structure and dynamics of the sediment (Johnson *et al.*, 2010; Harvey *et al.*, 2011). In particular, many species of crayfish are very active burrowers which can lead to extensive sediment resuspension and sediment restructuring (Davis, 1993; Barbaresi *et al.*, 2004b; Harvey *et al.*, 2011; Rice *et al.*, 2016). Furthermore, at least 20 species of crayfish have been introduced outside their native ranges, with many of these species now well established at multiple locations on two or more continents (Rodríguez & Suárez, 2001; Hänfling *et al.*, 2011). For example, *P. clarkii* has been extensively introduced around the world and is now widespread on all continents except Australia and Antarctica (Rodríguez & Suárez, 2001). Given the potential power of bioturbation as an ecosystem engineering process, the presence of invasive crayfish species in freshwater systems all around the world could be an important global driver of freshwater biodiversity loss and habitat degradation.

1.3 Potential ramifications of crayfish bioturbation

Despite the extensive research that has been done on invasive crayfish species, relatively few studies have actually investigated the impacts of crayfish bioturbation. Most studies on bioturbation in general have focused on either fish or on small invertebrates which both operate at different size scale to crayfish; consequently, it is relatively unclear to what extent invasive

crayfish bioturbation will impact recipient ecosystems. The studies that have investigated crayfish bioturbation have primarily focused on its direct impacts on the physical environment, thereby identifying the existence and mechanisms of crayfish bioturbation. It has been found that crayfish act as important geomorphic agents in freshwater systems by winnowing fine sediments from the bed (Parkyn *et al.*, 1997; Usio & Townsend, 2004; Harvey *et al.*, 2014) and burrowing into the bed and bank materials (Barbaresi *et al.*, 2004b) thereby destabilising banks (Guan, 1994) and increasing water column turbidity (Angeler *et al.*, 2001; Harvey *et al.*, 2014; Rice *et al.*, 2016). It has been estimated that a single medium sized signal crayfish could produce a sediment displacement of 1.7 kg m^{-2} d^{-1} (Johnson *et al.*, 2010). Given the huge bioturbation potential of crayfish, the knock-on or indirect impacts are likely to be many and varied; however, to date, the majority of studies on the indirect impacts of crayfish bioturbation have primarily focused on either sediment transport or nutrient dynamics. In this section I will use these works to discuss which other ecosystem properties and processes may be affected by crayfish bioturbation.

In the studies referenced above, crayfish have been found to be capable of significant disturbance of various sediment types, ranging from fine silts (Harvey *et al.*, 2014) to gravels (Johnson *et al.*, 2010). From these studies it is evident that the physical properties of the sediment are an important determinant of the extent to which it is affected by crayfish bioturbation. For example, bioturbation of fine sediments has been found to impact sediment resuspension, transport and topography (Harvey *et al.*, 2014), whilst bioturbation of gravels results primarily in topographical alterations only (Johnson *et al.*, 2010). As such, the nature and severity of any indirect biotic impacts of crayfish bioturbation will be intrinsically linked with local sediment characteristics. Given that invasive species, such as crayfish, are most frequently introduced to habitats in close proximity to human habitation (e.g. Jackson & Grey, 2013), it is likely that lowland rivers, ponds and lakes, which typically have abundant fine sediment, are the habitats most commonly impacted by invasive crayfish bioturbation. Consequently, since previous studies indicate that fine sediments are the most heavily affected by crayfish bioturbation, the rest of this section (and thesis) will focus on the potential indirect impacts of crayfish bioturbation of fine sediment in particular.

As mentioned above, crayfish bioturbation of fine sediment can result in sediment resuspension (Parkyn *et al.*, 1997; Usio & Townsend, 2004; Harvey *et al.*, 2014). Sediment resuspension typically promotes aerobic decomposition of suspended organic matter (Bastviken *et al.*, 2004) thereby increasing the biological oxygen demand of the water column and internal nutrient

loading (Angeler *et al.*, 2001). Increased nutrient concentrations (i.e. eutrophication) are well known to cause a variety of impacts that are usually viewed as deleterious (Table 1.1). Perhaps the most obvious effect of nutrient loading is the accumulation of nuisance levels of algal biomass, otherwise known as algal blooms (Smith *et al.*, 1999). Algal blooms occur because eutrophication releases phytoplankton from nutrient limitation, typically by Nitrogen (N) or Phosphorus (P), therefore enabling rapid population growth (Guildford & Hecky, 2000; Elser *et al.*, 2007). The ability of bioturbation induced nutrient loading to increase algal biomass has been experimentally demonstrated for the benthivorous fish *Dorosoma cepedianum* and postulated as the driving force behind the occurrence of algal blooms following *P. clarkii* invasion of lentic habitats (Angeler *et al.*, 2001; Rodríguez *et al.*, 2003). In addition, Yamamoto (2010) experimentally demonstrated that *P. clarkii* bioturbation can directly recruit bloom-forming cyanobacteria from the sediment into the water column, thereby compounding the effects of nutrient loading on algal biomass.

Table 1.1: Effects of eutrophication on freshwaters (modified from Smith, Tilman & Nekola, 1999)

Increased biomass of freshwater phytoplankton and periphyton
Shifts in phytoplankton species composition to taxa that may be toxic or inedible (e.g. bloom-forming cyanobacteria)
Changes in vascular plant production, biomass, and species composition
Reduced water clarity
Dense algal mats reduce habitat quality for macroinvertebrates and fish spawning
Decreases in the perceived aesthetic value of the water body
Taste, odor, and water supply filtration problems
Possible health risks in water supplies
Elevated pH and dissolved oxygen depletion in the water column
Increased fish production and harvest
Shifts in fish species composition towards less desirable species
Increased probability of fish kills

Numerous studies have shown that when algal blooms occur, dissolved oxygen (O_2) concentrations tend to decrease due to increased biological oxygen demand within the water column (e.g. Paerl & Otten, 2013). Given that suspended sediment can also increase oxygen demand (Bastviken *et al.*, 2004), the resuspension of sediment followed by algal blooms in response to crayfish bioturbation could drive O_2 concentrations down even lower than expected by an algal bloom or suspended sediment alone and potentially even maintain an oxygen deficit when blooms are not occurring. Changes in oxygen dynamics are obviously of concern to ecosystem functioning, since much of the food web relies on aerobic metabolism and so oxygen

depletion can significantly reduce biodiversity (Seitz *et al.*, 2009). The animals which are best known for their strong reaction to low oxygen are fish, with large scale fish kills often occurring in lakes where oxygen depletion has occurred due to pollution or eutrophication (Camargo & Alonso, 2006). This is of additional concern, since many freshwater fish, such as trout and roach, are of significant recreational or commercial value and so it is especially important to conduct further study into whether invasive crayfish bioturbation can cause sufficient oxygen depletion to cause ecological harm.

If any changes in decomposition occur in either the water column or the sediment as a result of crayfish bioturbation, it is expected that the production of decompositional by-products will also be altered (Angeler *et al.*, 2001); for example methane (CH_4), which is produced by anaerobic decomposition in the sediment. The dynamics of CH_4 in freshwaters have been of particular interest in recent years since freshwaters are now recognized as a major global source of this potent greenhouse gas. However, CH_4 dynamics in aquatic systems are controlled by more than just methanogenesis, since methane oxidation by methane oxidising bacteria (MOB) and ebullition can remove CH_4 from the system. Biogenic methane is generated in the deep anaerobic sediment and diffuses upwards along a concentration gradient. As CH_4 reaches the sediment-water boundary, the increasing oxygen concentration allows MOB to oxidise the CH_4 for use as an alternative carbon source. Since MOB require both CH_4 and O_2 for methane oxidation, they tend to mostly occur where both substrates are available in high concentrations, i.e. at the sediment-water interface. Given that bioturbation can cause significant disturbance of the sediment-water interface we might expect CH_4 dynamics to change as a result of bioturbation, but the exact nature of this change cannot be predicted (Figure 1.1). This issue is particularly interesting because recent research has shown that apart from its greenhouse gas properties, CH_4 can also be an important energy source in freshwater food-webs (Jones & Grey, 2011; Grey, 2016). It has even been estimated that net methanotrophy may be the equivalent of up to 46% of net benthic photosynthetic production in lowland chalk rivers (Shelley *et al.*, 2014), a habitat that is frequently invaded by signal crayfish, *P. leniusculus*. Therefore, a change in methane dynamics in response to crayfish bioturbation could affect energy flow through the food-web and hence alter ecosystem functioning. For instance, sediment resuspension may redistribute MOB from the sediment to the water column, where they may be more available as a food source to filter feeding organisms such as zooplankton. If so then zooplankton may incorporate more methane derived carbon into their biomass, much like chironomid larvae ingesting the bacterial colonies that grow inside their burrows (Jones & Grey, 2011). Furthermore, since zooplankton is a major food source for the pelagic food web, methane-

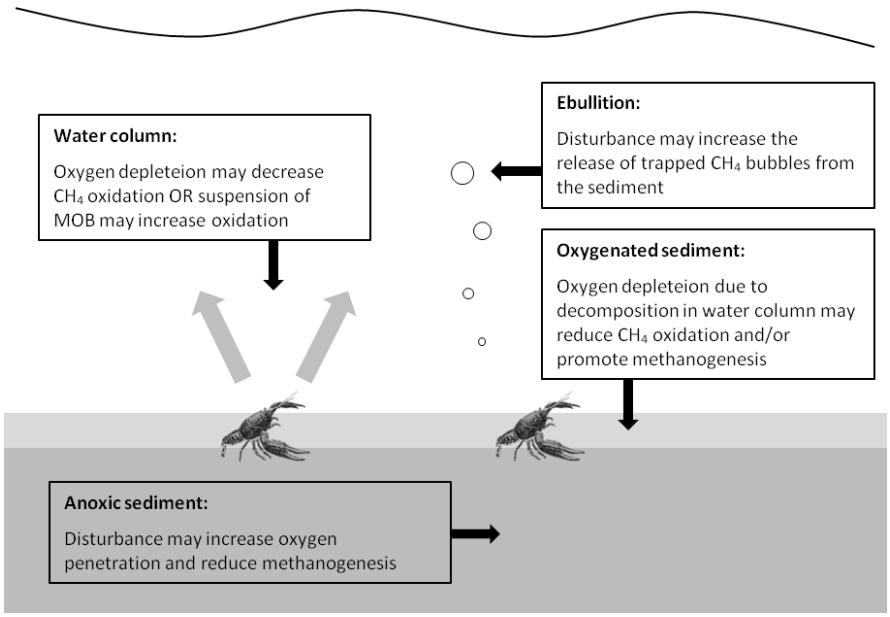

Figure 1.1: Possible effects of crayfish bioturbation on methane dynamics

derived carbon could become more prevalent throughout the entire food web in bioturbated waters.

Sediment resuspension and algal blooms are both very large perturbations to which the zooplankton community is known to respond. For example, Cottenie *et al.* (2001) found that turbid ponds with high algal biomass had increased abundances of rotifers and cyclopoid copepods relative to clear water ponds. It is therefore feasible that the zooplankton community may change in response to bioturbation by invading crayfish. However, the exact nature of any change in community structure is difficult to predict since it will likely depend on a variety of factors, such as suspended particle size, food resource composition, food web structure and time of year (Cottenie *et al.*, 2001). The concept of trophic cascades demonstrates that food webs can be extremely sensitive to perturbations (Carpenter *et al.*, 1985); therefore, changes in the relative abundance of a major food resource such as zooplankton could potentially cascade through the food web. For example, a shift towards larger species could advantage visually limited fish predators at the expense of gape limited invertebrate predators (Greene, 1983; Figure 1.2), thereby potentially changing their relative abundances and so altering ecosystem functioning.

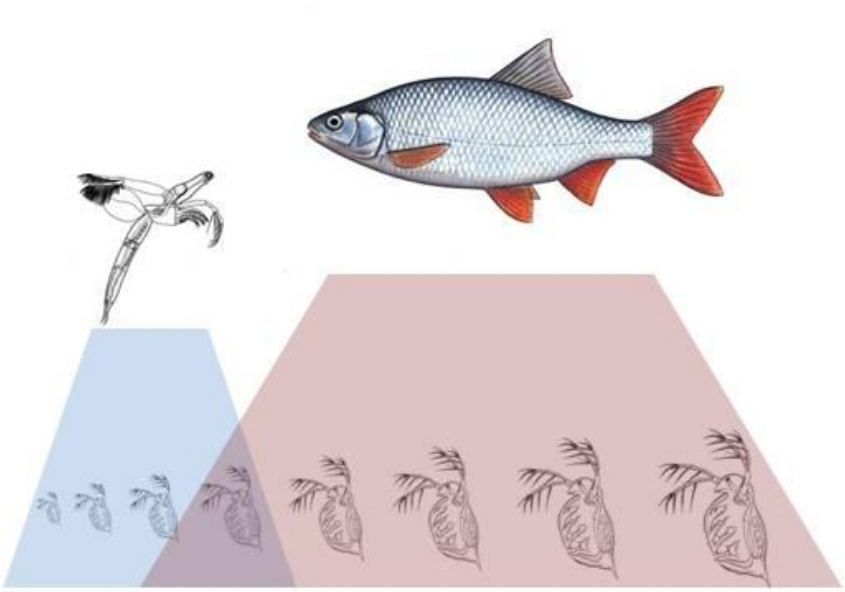

Figure 1.2: Simplified illustration of gape limitation in zooplanktivorous invertebrates and fish. Invertebrates predominantly consume smaller zooplankton than fish and so shifts in zooplankton size can impact food availability for either predator depending on the direction of change.

1.4 Differences in bioturbation activity between crayfish species

Thus far, discussion of crayfish bioturbation has ignored any possible variation in bioturbation between invasive crayfish species; however, different species are likely to have different patterns of activity or behaviours which result in differing degrees of bioturbation. It is well known that some invasive crayfish species such as signal, *P. leniusculus* and red swamp, *P. clarkii*, are extremely active burrowers (Guan, 1994; Barbaresi *et al.*, 2004b) whilst others such as Turkish, *Astacus leptodactylus*, have not been reported as such. Since bioturbation is by definition a direct effect of burrowing, it is likely that intensity of bioturbation will vary between these species. However, other activities such as foraging and walking will also induce bioturbation and thus potentially confound any effect of burrowing intensity, especially if the degree of bioturbation resulting from these activities also varies between species. Variation in bioturbation intensity resulting from similar activities may occur because different species have different average sizes and morphologies and so may interact with the sediment in different ways (Willis-Jones *et al.*, 2016). Finally, invasive crayfish species come from a wide variety of native environments, *P. clarkii* from warm subtropical wetlands (Hernandez *et al.*, 2008) and *P. leniusculus* and *A. leptodactylus* from more temperate waters (Holdich, 2002; Larson & Olden, 2011), therefore the pattern of seasonal bioturbation activity in response to temperature will probably vary for each species. Taken together, the differences in behaviour, ecology and biology between invasive crayfish species mean that it is not necessarily possible to extrapolate

our understanding of the impacts of crayfish bioturbation from one species to another. Therefore, given that there are numerous invasive crayfish species around the world it is necessary for comparative work to be done on several species to clarify this.

1.5 Experimental approach

This thesis aims to expand upon and further the work described above by answering the following questions:

1. *Does crayfish bioturbation promote an oxygen deficit and impact upon methane gas mediation?* Resuspension of organic matter should stimulate microbial metabolism and so reduce oxygen concentration. The greenhouse gas methane and its production, use and release are tightly coupled within the sediment and so disturbance could affect its emission to the atmosphere and/or its importance as an energy source for the food web.
2. *Are some invasive crayfish 'worse' than others in terms of question 1 above?* There are seven alien crayfish species in the UK with different patterns of diel and seasonal activity which could affect their bioturbation potential.

In order to address the research questions a single mesocosm experiment per crayfish species is not enough. Crayfish are poikilothermic and so their activity is likely to vary considerably over time (seasonally) as will responses to that activity by the rest of the community. Consequently it is necessary to repeat the mesocosm experiments at several different times of year to understand the true extent of any crayfish bioturbation impacts. However, given the turnaround time between each experiment it is only possible to obtain a maximum of 4 seasonal time points for a single crayfish species. Consequently it is also necessary to gain a specific understanding of how crayfish bioturbation activity responds directly to temperature so that effects observed at discrete time points throughout the year can be extrapolated to estimate a full annual cycle.

1.6 Mesocosms as the study system

Experimental mesocosms were chosen as the primary study system rather than "real world" systems because they allow a much higher degree of control and replication. In particular, it is extremely difficult to determine with any accuracy the population density of crayfish in a natural lake or river (Momot *et al.*, 1978) and so it would be difficult to determine the extent to which any observed effects derived from crayfish activity versus other variations between experimental sites. In addition, the use of mesocosms meant that variations of environmental and biotic factors between replicates such as sediment type and community structure were minimised so that replicates were highly comparable and treatment effects could be more

Figure 1.3: Experimental mesocosm setup, showing emplacement of turbidity and oxygen probes

readily discerned. Despite these advantages, it is a common concern that mesocosms do not realistically simulate natural ecosystems since they are small closed systems and so their results cannot be usefully applied to larger and more complex real world situations (Carpenter, 1996). This concern is legitimate to a certain degree and needs to be addressed when trying to interpret the results of a mesocosm experiment. However, the ability to test hypotheses that would be impossible to test in the field still make mesocosms a valuable tool for investigating various questions in aquatic ecology from interactions between newly invasive species (Jackson *et al.*, 2014) to the effect of climate warming on ecosystem carbon cycling (Yvon-Durocher *et al.*, 2010). In order to maximise the applicability of the results obtained from the mesocosm experiments to real world situations, the mesocosms were setup with animals, plants and sediment that would realistically be found together in a natural pond or lake. Additionally, each experiment was only run for a period of six weeks in order to minimise variations in community structure between ponds due to stochastic effects in small populations. Finally, an approach that has proved useful when interpreting other mesocosm experiments is to compare the results with data collected from a variety of appropriate natural systems (Ledger *et al.*, 2009; Brown *et al.*, 2011). By looking for the same relationships that have been previously characterised in the mesocosms it is then possible to understand their relative importance in a natural system.

This thesis will therefore also aim to answer the following question:

3. *Do results from mesocosm experiments scale up to real world situations?* – Mesocosms suffer from limited realism and so predictions based on their results need to be checked with real world data.

1.7 Structural outline of the thesis

The research presented in this thesis aims to address the three questions detailed above. The research is divided into four chapters, with Chapter 2 addressing Question 1, Chapters 3 and 4 addressing Question 2and Chapter 5 addressing Question 3; as outlined below:***Chapter 2: Indirect impacts of Procambarus clarkii bioturbation on ecosystem processes: a seasonal mesocosm study***

This chapter addresses Question 1 through use of 24 experimental mesocosms in which the density of a widespread crayfish species, *P. clarkii*, was controlled in order to manipulate the intensity of bioturbation activity and thereby identify relationships between crayfish bioturbation and a number of ecosystem properties and processes. Experiments were conducted at four different times of year (once each in winter, spring, summer and autumn) to identify the importance of seasonal variations on the relationships identified.

Chapter 3: Comparison of bioturbation impacts with other crayfish species

This chapter begins addressing Question 2 by repeating the mesocosm experiments with two other widespread crayfish species, *P. leniusculus* and *A. leptodactylus*, in spring and autumn, thereby allowing direct comparison of the impacts of bioturbation by each species under nearly identical conditions.

Chapter 4: The impact of temperature on crayfish bioturbation intensity and its use in extrapolation of mesocosm experimental results

In this chapter the relationship between the intensity of bioturbation activity and temperature is modelled for four widespread crayfish species, *P. Clarkii*, *P. leniusculus*, *A leptodactylus* and *O. Virilis*. These models are used to address Question 2 by extrapolating the results of the previous chapters to generate predictions of the pattern of bioturbation intensity throughout the entire year for each species, which are then compared between species.

Chapter 5: Effect of manipulation of crayfish density on bioturbation impacts in a lowland chalk stream

In this chapter question 3 is addressed through use of a field experimental approach to manipulate the population density of *P. leniusculus* in three adjacent stretches of a lowland chalk stream. This setup was used to investigate the relationships between crayfish density, turbidity, dissolved oxygen concentration and methane oxidation in a real world situation.

Chapter 2: Indirect impacts of *Procambarus clarkii* bioturbation on ecosystem processes: a seasonal mesocosm study

2.1 Introduction

The red swamp crayfish, *Procambarus clarkii*, is native to the South Central United States and Mexico where it is commonly found in warm, turbid, slow-flowing waters such as the bayous of Louisiana, USA (Hobbs & Lodge, 2010). Despite its sub-tropical origin, it is perhaps the most widely introduced crayfish species in the world (Lodge *et al.*, 2012). It is considered invasive on five continents and can be found from equatorial Kenya to the temperate United Kingdom. Given its (unnaturally) wide distribution, much study has focused on the impacts of *P. clarkii* on the ecosystems into which it is introduced. However, most of these studies have focused primarily on the direct impacts of these crayfish on the community and food web structure of their recipient ecosystems.

The biotic impacts of *P. clarkii* have been mainly attributed to their highly omnivorous feeding habits, which allow them to directly change the abundance of species across the entire food web including amphibians, fish, gastropods, insect larvae and macrophytes (Renai & Gherardi, 2004; Geiger *et al.*, 2005; Gherardi, 2006; Gherardi & Acquistapace, 2007). This highly generalist feeding strategy combined with the potential to reach high population densities can lead to dramatic reductions in desirable taxa such as macrophytes and cause a major change in community structure and functioning (Rodríguez *et al.*, 2003; Geiger *et al.*, 2005). In addition, this canalises energy flow through the food web by increasing connectivity between different trophic levels in the aquatic (Geiger *et al.*, 2005) and even terrestrial food web, since this species has also been found to graze nocturnally on grass (Grey & Jackson, 2012), thereby reducing food web complexity. *P. clarkii* is known to carry the crayfish plague, Aphanomyces astaci, which in combination with increased competition has the potential to cause the local extinction of native crayfish species (Cioni & Gherardi, 2004; Gil-Sánchez & Alba-Tercedor, 2006; Holdich *et al.*, 2009).

In recent years, there has been increased focus on the impact of *P. clarkii* on the physical environment. It has been found that *P. clarkii* can cause the erosion of littoral zone sediments, changing benthic geomorphology (Angeler *et al.*, 2001) and increase bank erosion with the potential for significant economic and ecological impacts (Barbaresi *et al.*, 2004b; Anastácio, P. M. *et al.*, 2005). However there has been relatively little work done on the indirect knock-on

impacts of this bioturbation on the recipient ecosystems. What studies there are show that *P. clarkii* increases suspended solids through activities such as burrowing (Angeler *et al.*, 2001; Barbaresi *et al.*, 2004b), whilst the removal of macrophytes reduces the rate at which suspended sediment settles back out (Rodríguez *et al.*, 2003). In addition, *P. clarkii* bioturbation has also been linked with an increase in the rate of nutrient release from the sediment into the water (Angeler *et al.*, 2001). Taken together, these studies indicate the potential for *P. clarkii* to trigger a shift from a clear water state to a turbid, algae dominated state, as has been documented for a Spanish lake following *P. clarkii* invasion (Rodríguez *et al.*, 2003). The common feature of these studies is that *P. clarkii* produces significant increases in turbidity in the systems it invades but that relatively little is known about the wider impacts of this.

Despite the lack of previous study, it is possible to hypothesise (based on discussion here and in chapter 1) a variety of ecosystem properties and processes that may be affected by *P. clarkii* bioturbation:

1. Each individual crayfish undertakes its own bioturbation activities, such as burrowing, walking and feeding, thus overall bioturbation should be cumulative and increase with population density.
2. Decomposition of organic matter in the sediment is limited by the availability of oxygen. Thus, suspension of sediment in the comparatively well oxygenated water column should accelerate decomposition, reducing dissolved oxygen concentrations.
3. Accelerated decomposition should also increase nutrient cycling and thereby alter water chemistry and have a positive impact on algal biomass.
4. Changes in dissolved oxygen, water chemistry, algal biomass and macrophyte coverage should cause changes in community structure, especially for environmentally sensitive primary consumers such as zooplankton.
5. Methane production, utilisation and release are tightly coupled with sediment structure. Thus, sediment disturbance may alter methane dynamics (see Figure 1.1).
6. Methane oxidising bacteria (MOB) are mostly found in surface sediment and are limited by dissolved oxygen concentrations. Thus sediment suspension should also result in suspension of MOB in the comparatively well oxygenated water column and so increase rates of methane oxidation.
7. Elevated numbers of MOB in water column may result in increased ingestion of MOB by filter feeding organisms such as zooplankton thereby increasing the importance of

methane derived carbon to the pelagic food web. This should be seen by a shift in carbon stable isotope values.

Given hypothesis 1, it was the object of the study detailed in this chapter to utilise a mesocosm experiment to generate a gradient of crayfish bioturbation by manipulating crayfish density in order to test the various hypothesised impacts of *P. clarkii* bioturbation in shallow still water habitats, which are those most frequently invaded by crayfish (Ruokonen *et al.*, 2012). It is unlikely that the relationship between bioturbation intensity and crayfish density is linear since agonistic behaviour, such as fighting, typically increases with population density (Savolainen *et al.*, 2004; Davis & Huber, 2007) and so bioturbation per crayfish may increase along with density. However, since this study only requires that a range of turbidities be produced, the exact nature of this relationship is not important. The study was designed as a series of short-term experiments that could be repeated at four different time points throughout of the year to investigate how *P. clarkii* impacts may vary seasonally. Since most studies have primarily focused on periods of peak activity, this seasonal approach gives a better understanding of the long-term impacts of *P. clarkii* invasion in strongly seasonal places such as the UK where *P. clarkii* is expected to expand its range in the coming decades (Ellis *et al.*, 2012).

2.2 Methods

2.2.1 Experimental setup

The mesocosms were fibreglass ponds with a volume of ≈277 L and sediment surface area of ≈0.5 m^2 (Figure 1.2) situated on the roof of the Fogg Building at Queen Mary University of London, Mile End campus. Before each experiment, the habitat and the lower tiers of a food web were created in an identical way in each mesocosm. Each replicate mesocosm (n = 24) contained six evenly spaced bricks (215x65x102 mm) to provide contrasting substrate to that of natural pond sediment which was supplied to a depth of 70mm surrounding the bricks. The sediment comprised mostly of fine particulate matter with approximately 10% gravel. Coarse organic matter was maintained in the sediment through the addition of leaf litter (1x 5L bucket/ mesocosm) before the start of each experiment. In addition, before each experiment the sediment from all the mesocosms was combined, thoroughly mixed and redistributed to ensure that the sediment characteristics remained identical in each mesocosm. The mesocosms were filled with tap water and left to stand in full sunlight for one week to allow for complete degassing of residual chlorine. Approximately one week before the beginning of an experiment, the mesocosms were each seeded with three sprigs of Canadian pond weed, *Elodea canadensis*,

and a concentrated mix of zooplankton and aquatic invertebrates from a nearby pond. Between each experiment the mesocosms were drained and all the sediment was removed and thoroughly re-mixed and redistributed.

The start of each experiment was marked by the addition of three crayfish treatments to the mesocosms: zero crayfish (controls); two crayfish (4 individuals m^{-2} equivalent to low-medium density); and four crayfish (8 ind m^{-2} equivalent to high density), reflecting densities of *P. clarkii* that have been reported in the field (Momot *et al.*, 1978; Gherardi & Lazzara, 2006; Chucholl, 2013). These numbers also allowed a constant sex ratio of 1:1 to be maintained across both treatments, thereby minimising variation in activity due to behaviour between the mesocosms. All the crayfish used were adults, had intact and equal chelae and carapace lengths of 40-70mm. The mesocosms were regularly checked for dead crayfish, which were immediately removed and replaced with an appropriate individual of same size and sex. Each experiment was run for six weeks, after which all data was collected.

2.2.2 Turbidity

The turbidity of the water was measured every 5 minutes for 24 hours with 6 turbidity probes (Partech IR40C probes connected to a Campbell Scientific CR1000 data logger), with a single probe suspended 10cm below the water surface in the centre of each mesocosm. Since all 24 mesocosms could not be measured simultaneously in this way, the mesocosms were split into eight blocks of three (one of each crayfish density treatment per block), with two blocks being measured per day over four consecutive days. The probes were washed in clean water after removal from each mesocosm to reduce soiling of the optical lenses and to minimise cross-contamination between the mesocosms, The blocks were assigned geographically, so that mesocosms with similar environmental conditions such as light or temperature regime (variable between mesocosms due to intermittent shading from nearby structures) were sampled simultaneously, thereby allowing any such variability to be accounted for during statistical analysis. The probes were calibrated at 10 points with formazin FTU standards to give a sigmoidal calibration curve which was fitted with a third order polynomial function.

2.2.3 Dissolved Oxygen

Dissolved oxygen was measured simultaneously with, and at the same rate as, turbidity with Unisense OX-10 oxygen microsensors attached to a Unisense UnderWater data logger. Due to equipment failures, data were only collected for fifteen mesocosms in the spring and 18 mesocosms in the summer. However, these missing data values do not significantly affect

analysis of the results as data were still collected at a wide range of turbidities and the statistical methods used for analysis are robust against the slight imbalance this created in the data.

2.2.4 Dissolved Methane

One water sample from each mesocosm was collected at 1200 midday (to avoid changes from diurnal fluctuation) on three different days from ≈10 cm below the surface with a gas-tight 50 ml syringe. The water was then discharged into a gas-tight 12.5 ml glass vial until overflowing and then capped. All samples had a 2ml helium headspace introduced and were shaken for 5 minutes before being immediately analysed by gas chromatography with flame ionising detection (GC/FID; Agilent Technologies UK Ltd., South Queensferry, U.K.; Sanders *et al.*, 2007)

2.2.5 Methanogenic & Methane Oxidation Potentials

Methane oxidation potentials of the water column (MOP_{wat}) and sediment (MOP_{sed}) were measured in all experimental runs, whilst methanogenic potential in the sediment (MGP) was measured in all except the winter run. MGP was not measured in the winter as it was the first experimental run and it was not decided to measure MGP until after that experiment had concluded. For the MOP_{wat}, water samples were collected from each mesocosm in the same manner as for the dissolved CH_4, except only 6 ml of water were discharged into the 12.5 ml vials, with the remainder of the volume occupied by an ambient air headspace. For MGP and MOP_{sed} a single sediment core sample was taken from each mesocosm using a truncated 5 ml syringe. Approximately 0.5 ml of sediment was taken from both the top and bottom of each core (for MOP_{sed} and MGP respectively) and placed into pre-weighed 12.5 ml glass vials. Into each of these vials, 3 ml of water from the appropriate mesocosm was added to better simulate an aquatic environment. The MOP_{sed} vials were then sealed to leave a 9 ml ambient air headspace, whilst the MGP vials were capped and flushed with nitrogen for 10 minutes to remove all oxygen. Both sets of MOP vials were enriched with methane to a target aqueous concentration of 2 µmol L^{-1} and shaken for 5 minutes. CH_4 in all vials was immediately analysed by GC/FID to determine its concentration at t=0. Vials were then incubated on shakers at 22-24°C and were analysed on at least 3 further time points to determine the rate of increase or decrease of CH_4 (Sanders *et al.*, 2007). After the final time point, the sediment vials were dried to constant weight at 60°C and reweighed to determine the mass of sediment, so that rates of methane oxidation and methanogenesis could be normalised for dry mass.

Whilst all MOP vials were enriched to a target concentration of 2 µmol L^{-1}, there were variations in the initial concentration actually achieved, probably due to methane already present in the

water. However, previous work has shown a positive linear relationship between initial methane concentration and the rate of methane oxidation (Shelley *et al.*, 2014). To control for this, incubations were set up as described for MOP_{wat} above, with water from several mesocosms but with varying spikes of methane to create aqueous methane concentrations ranging from 0-4 µmol L^{-1}, thereby encompassing the complete range of methane concentrations measured in the MOP vials. This linear relationship was then used to normalise all methane oxidation potentials to a methane concentration of 2 µmol L^{-1}.

2.2.6 Chlorophyll a

In the final week of each experimental run, approximately 500 ml samples of the entire water column were taken from each mesocosm with a 4 cm diameter plastic tube. 200 ml (winter, spring and autumn) or 100 ml (summer) subsamples were taken from each sample and vacuum filtered through Whatman GF/C filters. A smaller volume was filtered in the summer since large quantities of suspended sediment in some mesocosms prevented filtering of more than 100 ml; however, higher algal biomass meant that this volume was still sufficient to obtain reliable results. The filters were folded in aluminium foil and stored at -18°C for 1-2 weeks to await analysis. Filters were then immersed in 5 ml of 90% acetone at 4°C in darkness for 24 hours, after which the filters were removed and the absorption of light at 665nm and 750nm was measured for each acetone sample. The chlorophyll *a* concentration was then estimated in µg L^{-1} using the Lorenzen monochromatic formula as per Dalsgaard et al (2000). No acidification to correct for the presence of phaeopigments was undertaken since only a relative measure of algal biomass per mesocosm was required for comparison between the mesocosms.

2.2.7 Nutrients

15 ml of the excess water samples collected for chlorophyll analysis were vacuum filtered through Whatman GF/F filters. The filtrate was stored at -18°C until analysis, whereupon the samples were thawed and the concentrations of nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3) and total phosphate (P) were measured with a Skalar San^{++} Continuous Flow Analyser.

2.2.8 Conductivity

Conductivity was measured with a Lovibond Sensodirect Con 200 handheld meter at the end of the spring and summer run. On each sampling occasion three measurements were taken from different locations in each mesocosm.

2.2.9 Zooplankton

After the collection of all other samples, the water column in each mesocosm was thoroughly mixed and 3 L of water was extracted using a 4 cm diameter plastic tube. The water was passed through a 180 µm mesh sieve and zooplankton was preserved in 10 ml of 95% ethanol. All individuals in a 1 ml sub-sample of the well-mixed preserved solution were identified to the lowest taxonomic grouping possible. Seven taxa were identified: *Daphnia magna, Daphnia pulex, Simnocephalus vetulus, Ceriodaphnia* spp*., Chydorus* spp*., Cyclops* spp. and *Ostrocods.* From these scores, the number of individuals L^{-1} was calculated for each taxon in each mesocosm.

For stable isotope analysis, 30-50 *D. magna* individuals were picked out from a fresh sample collected as above except without mixing of the water column. In addition, a 2 ml sample of surface sediment was collected from each pond using a 5 ml plastic syringe. The daphniids were left in 100 ml of clean ISO-water overnight to encourage gut clearance and were then rinsed three times in de-ionised water (*sensu* Feuchtmayr & Grey, 2003). Daphniids and sediment samples were dried at 60°C and then subsamples (at least 0.5 mg) were placed in tin cups and combusted using an elemental analyzer coupled to a continuous flow isotope ratio mass spectrometer (CF/IRMS, Thermo-Finnigan, Delta Matt Plus) for measurement of stable carbon isotopes (*sensu* Trimmer *et al.*, 2009).

2.2.10 Data Analysis

Analysis of seasonal patterns was done with a sinusoidal linear mixed effects model with the generic formula in Equation 1. A mixed effect model was used to incorporate random effects generated by the geographical blocking structure. The sinusoidal model was adopted since seasonal patterns typically follow a sinusoid pattern and the generic three parameter model in Equation 1 provided the best fit first approximation model. All seasonal models included crayfish density as an explanatory variable and interaction terms with all seasonal parameters. Models were refined by removal of non-significant factors to achieve the most parsimonious model. The final models were assessed by ANOVA with type III Sums of Squares.

$$\text{Equation 1:} \qquad y = \cos\left(\frac{2\pi}{12} \times \text{month}\right) + \sin\left(\frac{2\pi}{12} \times \text{month}\right) + \sin\left(\frac{4\pi}{12} \times \text{month}\right)$$

For analysis of turbidity and dissolved oxygen, measures of central tendency were taken for each mesocosm for both datasets; the median was used for turbidity since several of the high density treatments were skewed by occasional spikes in turbidity, whilst the mean was used for dissolved oxygen. All relationships within each experimental run were assessed with linear

models using type III Sums of Squares (oxygen analysis included a blocking factor). The dissolved CH_4, chlorophyll, and nutrient factors were transformed with natural logarithms to prevent violation of the linear model assumptions. Zooplankton communities were compared between treatments with MDS ordination techniques and analysed by PERMANOVA. All zooplankton scores were transformed with square roots to prevent the marginalisation of rare species. All statistical analyses were conducted using R statistical software (R Development Core Team 2017).

2.3 Results

2.3.1 Turbidity

Turbidity showed a seasonal pattern across all treatments (Figure 2.1). The sinusoidal seasonal model fitted the data very well (conditional R^2 = 0.99) and showed a significant interaction between crayfish treatment and all seasonal parameters (cos 2π: $F_{2,77}$ = 20.6, p < 0.001; sin 2π: $F_{2,77}$ = 28.1, p < 0.001; sin 4π: $F_{2,77}$ = 6.34, p < 0.01), indicating that seasonal fluctuations in turbidity varied in accordance with crayfish density. In particular, the presence of crayfish appeared to have no effect on turbidity in the autumn ($F_{2,21}$ = 1.97, p = 0.16) and winter ($F_{2,21}$ = 1.73, p = 0.20) months but during the spring and summer, higher crayfish densities were accompanied by higher turbidities (spring: $F_{2,21}$ = 17.4, p < 0.001; summer: $F_{2,21}$ = 16.2, p < 0.001), with the highest differences in turbidity being recorded during the summer experiment (High density mean (± S.E): 279 ± 28 FTU; Control mean (± S.E): 37 ± 14 FTU) .

The seasonal pattern in turbidity correlates well with the expected seasonal pattern of crayfish activity and therefore the turbidity of each mesocosm is probably a good measure of the crayfish bioturbation occurring within. Furthermore, given that each mesocosm receiving a crayfish addition (treatment) only contained a small number of crayfish, it is possible that variations in behaviour between individual crayfish could have had a significant impact on the amount of bioturbation that occurred in each mesocosm. Consequently, turbidity was used as the primary measure of bioturbation against which all other factors were analysed, except for assessment of seasonal patterns which retained the use of crayfish density as the measure of bioturbation since the use of a discrete variable that is not correlated with the seasonal variable makes interpretation of the model simpler.

2.3.2 Dissolved Oxygen

This sinusoidal seasonal model produced a good fit (conditional R^2 = 0.64) and showed a significant interaction between crayfish treatment and one seasonal parameter (cos 2π: $F_{2,65}$ = 9.79, $p < 0.001$), indicating that seasonal fluctuations in mean dissolved oxygen concentrations were greater in mesocosms with higher bioturbation (Figure 2.2A). In particular, there was no significant relationship between crayfish bioturbation and mean dissolved oxygen concentrations in the autumn ($F_{1,22}$ = 0.80, p = 0.38) or winter ($F_{1,13}$ = 1.42, p = 0.25) experiments but during the spring and summer, greater bioturbation was associated with a decrease in mean dissolved oxygen concentrations (spring: $F_{1,22}$ = 9.43, $p < 0.01$; summer: $F_{1,16}$ = 23.3, $p < 0.001$; Figure 2.2B). During the summer, the mean dissolved oxygen concentrations of the most turbid (high density treatment) mesocosms (177 ± 22 μmol L^{-1}) bordered on hypoxia (dissolved O_2 concentration below 125-190 μmol L^{-1}), meaning that organisms within these mesocosms would have experienced periods of hypoxic stress overnight when photosynthesis was not occurring, whilst control mesocosms maintained a mean concentration of 321 ± 21 μmol L^{-1}.

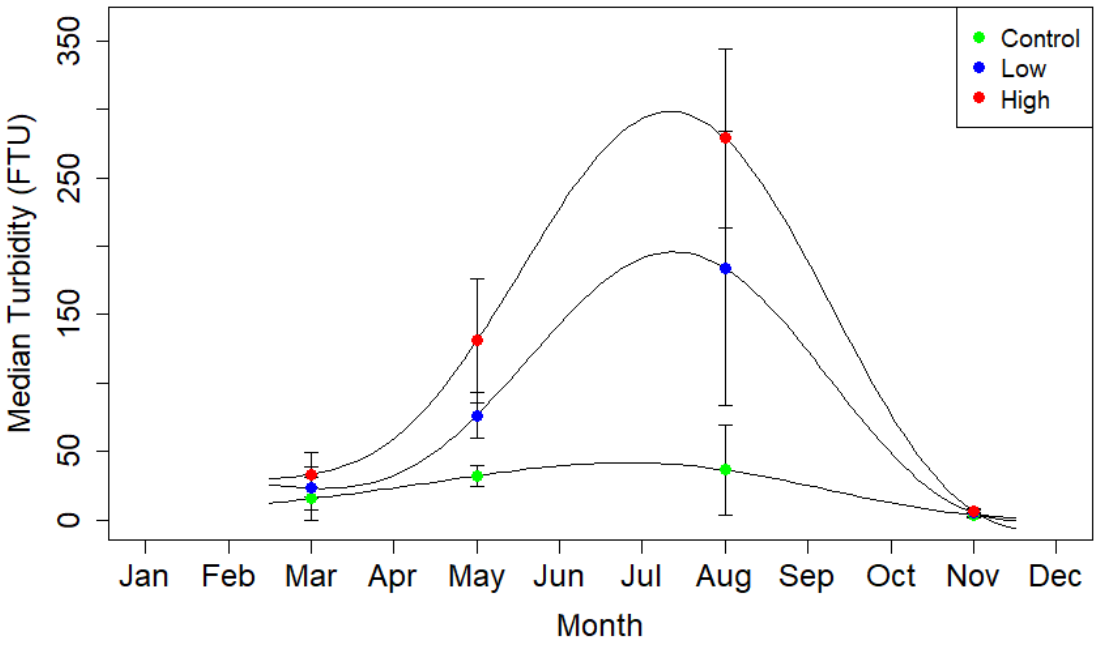

Figure 2.1: Seasonal variation in mesocosm turbidity.
Red circles represent high crayfish density treatment (n = 8), blue circles are low crayfish density treatment (n = 8), and green circles are the control treatment (n=8). Error bars show 95% confidence intervals. Solid lines show fit of sinusoidal seasonal model for each treatment.

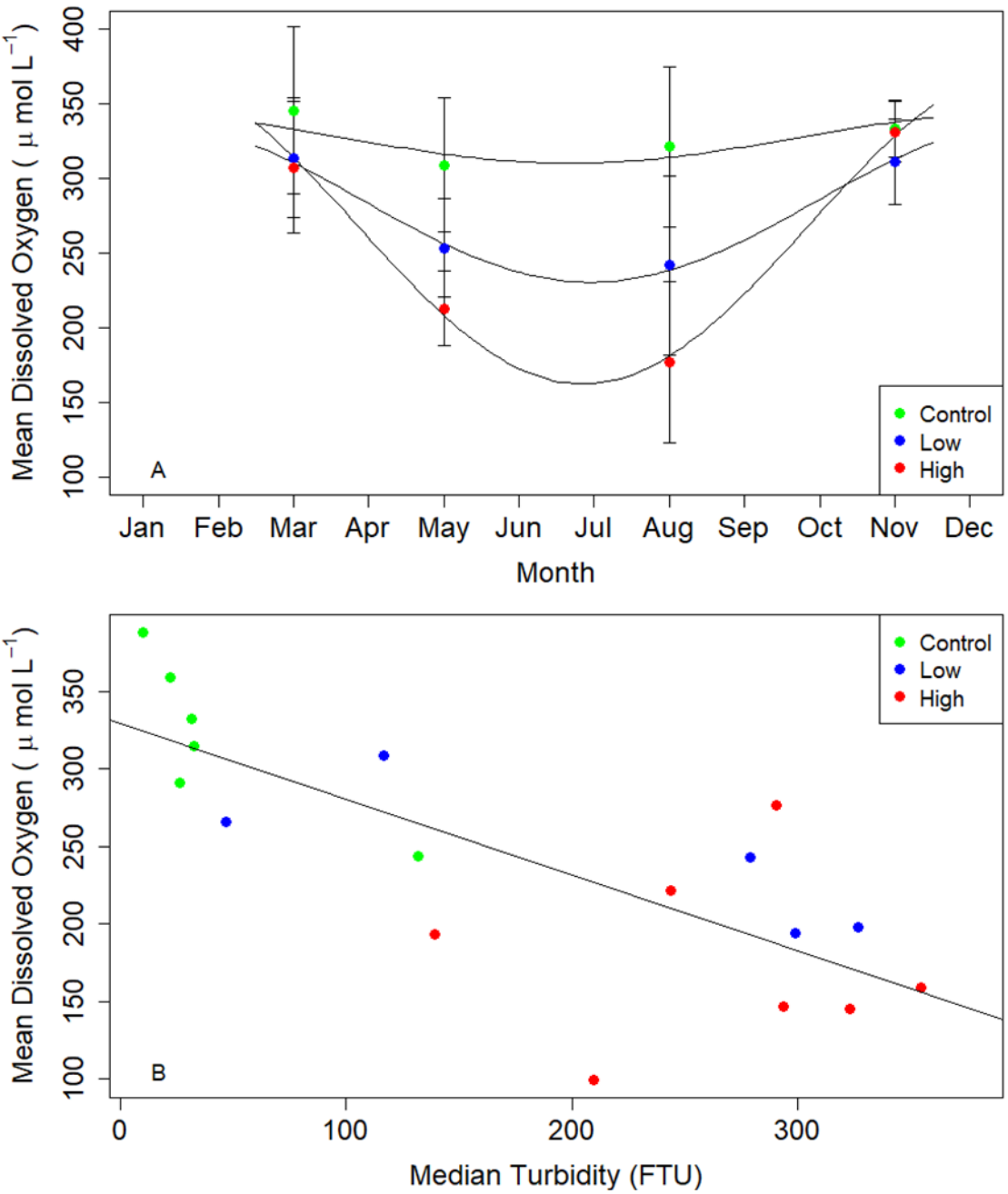

Figure 2.2: Seasonal variation in mesocosm dissolved oxygen concentrations and the relationship between dissolved oxygen and turbidity in the summer.

A) Mean (± 95% CI) dissolved oxygen concentrations in mesocosms across the year. Red circles are high crayfish density treatment (n = 8), blue circles are low crayfish density treatment (n = 8) and green circles are the control treatment (n = 8). Solid lines show fit of sinusoidal seasonal model for each treatment. B) Linear relationship between bioturbation (measured as turbidity); differing colour data-points reflecting crayfish density treatments as in A) and mean dissolved oxygen concentrations in the summer experiment (n = 18, R^2 = 0.57). Solid line is a fitted linear relationship with formula y = -0.49x + 329.5.

2.3.3 Chlorophyll a

Chlorophyll *a* concentrations were strongly seasonal with the largest difference in concentrations between the high density (102 ± 21 mg L^{-1}) and control (15 ± 2 mg L^{-1}) treatments being observed in the summer. The sinusoidal seasonal model provided a strong fit (conditional R^2 = 0.87) and showed a significant interaction between crayfish density and one seasonal parameter (sin 2π: $F_{2,63}$ = 7.78, p <0.01), indicating that seasonal fluctuations in algal biomass were greatest in mesocosms with the most bioturbation (Figure 2.3). In particular, there was a positive linear relationship between bioturbation and algal biomass in both the spring ($F_{1,22}$ = 4.98, p < 0.05) and summer ($F_{1,22}$ = 65.8, p < 0.001) experiments.

2.3.4 Nutrients and conductivity

Nutrient concentrations and conductivity were only measured in two experimental runs (nutrients: winter & summer runs; conductivity: spring & summer runs) and so no seasonal analysis was possible. No significant relationship was found between bioturbation and any nutrient concentrations in the winter, whilst in the summer, NH_3 concentration was found to have a strong positive relationship with bioturbation ($F_{1,22}$ = 39.1, p < 0.001). Conductivity was positively related with bioturbation in both the spring ($F_{1,22}$ = 13.1, p < 0.01) and summer ($F_{1,22}$ = 31.1, p < 0.001) experiments.

2.3.5 Methanogenesis, dissolved methane, and methane oxidation

Methanogenic potential (MGP) showed a seasonal relationship with the highest rates of methanogenesis being observed during the summer experiment. Crayfish density was found to have no significant relationship with methanogenesis ($F_{2,14}$ = 1.38, p = 0.28); however, the sinusoidal seasonal model did not provide a very good fit to the data (conditional R^2 = 0.006) indicating that the data were too variable to draw any meaningful conclusions.

Dissolved methane concentrations were oversaturated relative to atmospheric equilibration (3.2 nmol L^{-1} at 10°C) with a mean of approximately 2 µmol L^{-1} in spring and summer, and 1 µmol L^{-1} in autumn and winter (Figure 2.4). Crayfish density was found to have no significant explanatory power ($F_{2,14}$ =0.03, p = 0.97) and so was removed from the model, leaving month of the year as the only predictor for methane concentration. This indicates that crayfish bioturbation had no detectable impact on dissolved methane concentrations.

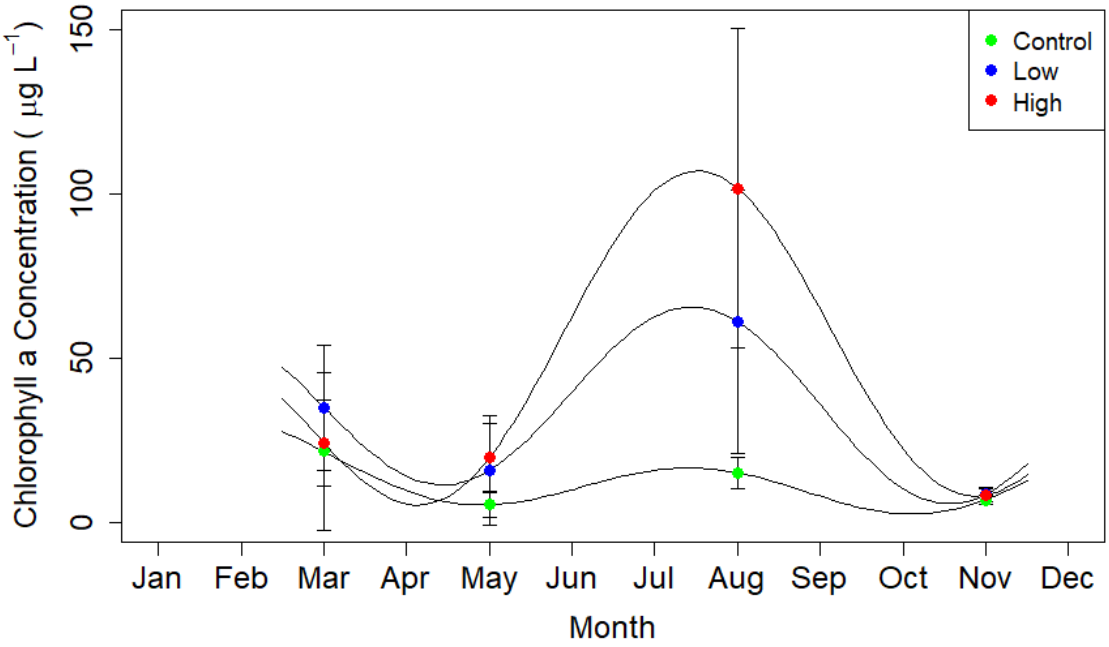

Figure 2.3: Seasonal variation in mesocosm chlorophyll *a* concentrations.
Mean (n = 8, ± 95% CI) chlorophyll *a* concentrations for each crayfish density treatment across the year: red is high density, blue is low density, and green is control. Solid lines show fit of sinusoidal seasonal model for each treatment.

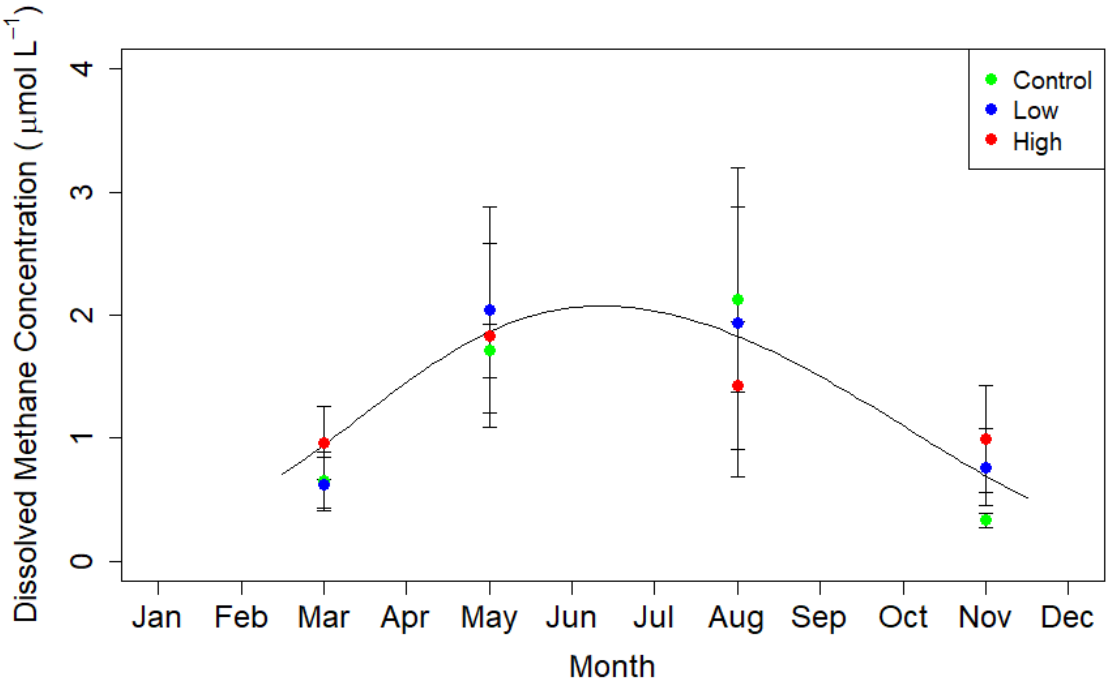

Figure 2.4: Seasonal variation in mesocosm dissolved methane concentrations.
Mean (n = 8, ± 95% CI) dissolved methane concentrations for each crayfish density treatment across the year: red is high density, blue is low density, and green is control. Solid line shows fit of sinusoidal seasonal model for all treatments combined since crayfish density treatment was found to have no explanatory power.

Methane oxidation potential of the water column (MOP_{wat}) showed a seasonal pattern across all treatments (Figure 2.5A), to which the sinusoidal seasonal model provided a good fit (conditional R^2 = 0.51). There was a significant interaction between crayfish treatment and two seasonal parameters (cos 2π: $F_{2,79}$ = 3.22, $p < 0.05$; sin 2π: $F_{2,79}$ = 11.5, $p < 0.001$) indicating that seasonal fluctuations in MOP_{wat} were greater in mesocosms with higher bioturbation. Significant positive asymptotic relationships between MOP_{wat} and bioturbation were observed in the spring and summer (Figure 2.5B) with the largest difference in mean methane oxidation rates between the high density (1.2 ± 0.04 μmol L^{-1} h^{-1}) and control (0.6 ± 0.1 μmol L^{-1} h^{-1}) treatments being observed during the summer experiment. Methane oxidation was clearly substrate limited, with a linear increase in rate with methane concentration ($F_{1,10}$ = 2052, $p < 0.001$; Figure 2.5C). Consequently this linear relationship was used to normalise all methane oxidation rates to a methane concentration of 2 μmol L^{-1} (the approximate mean concentration in both the spring and summer experiments) to maximise comparability of absolute methane oxidation potential between mesocosms and experiments.

Methane oxidation potential of the surface sediment (MOP_{sed}) showed a seasonal pattern across all treatments (Figure 2.6) with the sinusoidal seasonal model providing a very strong fit (conditional R^2 = 0.99). There was a significant interaction between crayfish density and one seasonal parameter (sin 4π: $F_{2,81}$ = 3.59, $p < 0.05$), indicating that seasonal fluctuations in MOP_{sed} were slightly smaller in mesocosms with more bioturbation. In particular, in the summer experiment there was a negative relationship between MOP_{sed} and bioturbation activity ($F_{1,22}$ = 5.14, $p < 0.05$); however, this weak relationship had R^2 = 0.15 and slope <0.001, suggesting that it is not particularly meaningful.

2.3.6 Zooplankton $\delta^{13}C$

The zooplankton $\delta^{13}C$ values showed a seasonal pattern for which the sinusoidal seasonal model provided a relatively good fit (conditional R^2 = 0.46). Crayfish density interacted significantly with one seasonal parameter (sin 2π: $F_{2,42}$ = 10.3, $p < 0.001$), indicating that seasonal fluctuations in zooplankton $\delta^{13}C$ were largest in the most turbid mesocosms (Figure 2.7A). The summer experiment showed a significant lowering of zooplankton $\delta^{13}C$ with bioturbation ($F_{1,22}$ = 40.6, $p < 0.001$; Figure 2.7B) and a strong linear relationship with water column methanotrophy ($F_{1,22}$ = 47.4, $p < 0.001$; Figure 2.7C). The mean sediment $\delta^{13}C$ was -24.4 ±0.18 ‰ (SEM, n=24.) Summer $\delta^{13}C$ values ranged from -26.2 to -15.5 ‰.

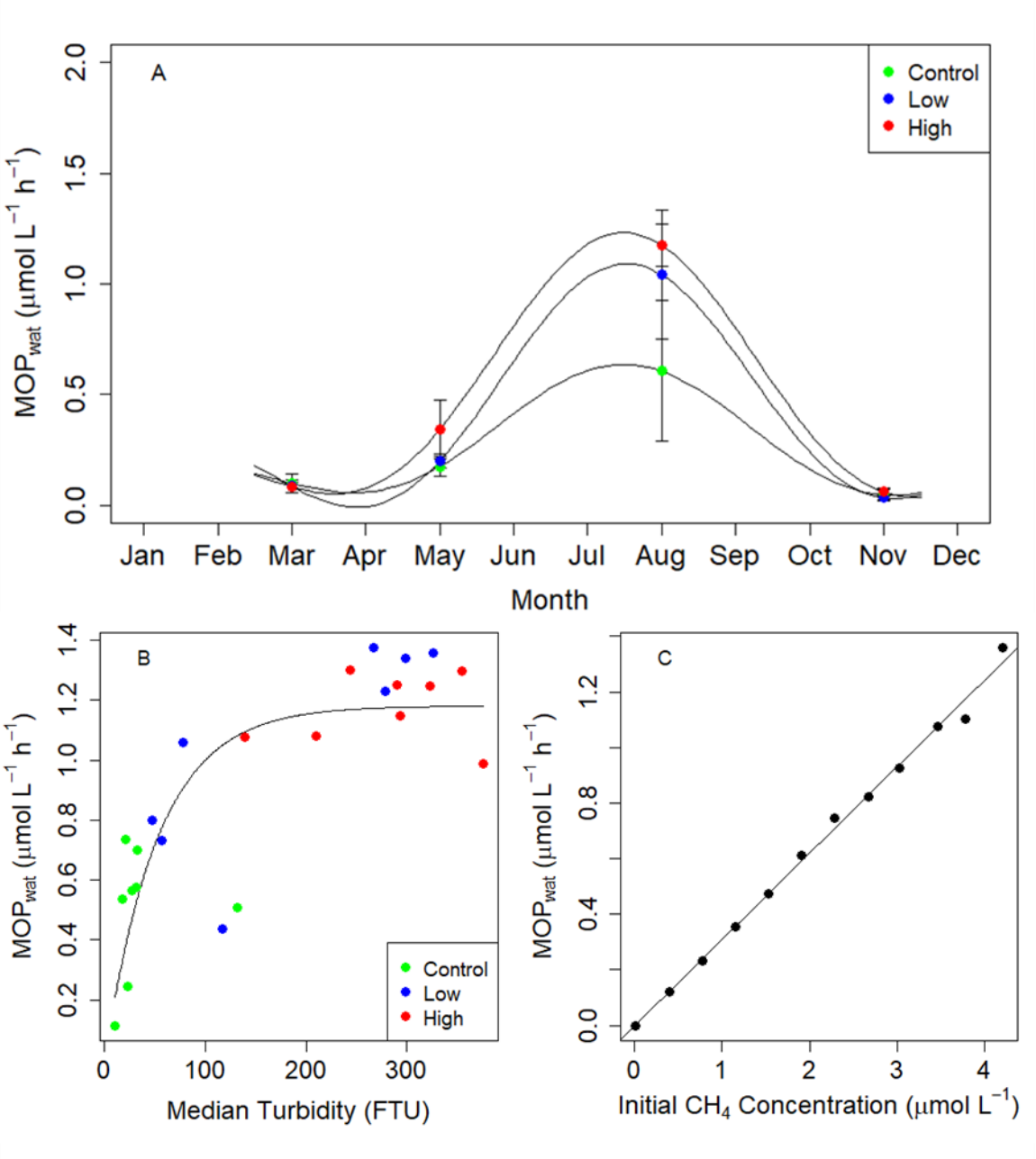

Figure 2.5 Seasonal variation in and factors influencing mesocosm methane oxidation potentials of the water column.

A) Mean (n = 8, ± 95% CI) methane oxidation potentials of the water column for each treatment across the year. Red is high density, blue is low density and green is control treatment. Solid lines show fit of sinusoidal seasonal model for each treatment. B) Shows the linear relationship between bioturbation activity, measured as turbidity, and methane oxidation potential of the water column in the summer experiment (n = 24, R^2 = 0.66, y = 0.0024x + 0.51). C) Shows the linear relationship between rate of methane oxidation and the methane concentration at the start of the incubation (n = 12, R^2 = 0.99, y = 3.2x + 0.012).

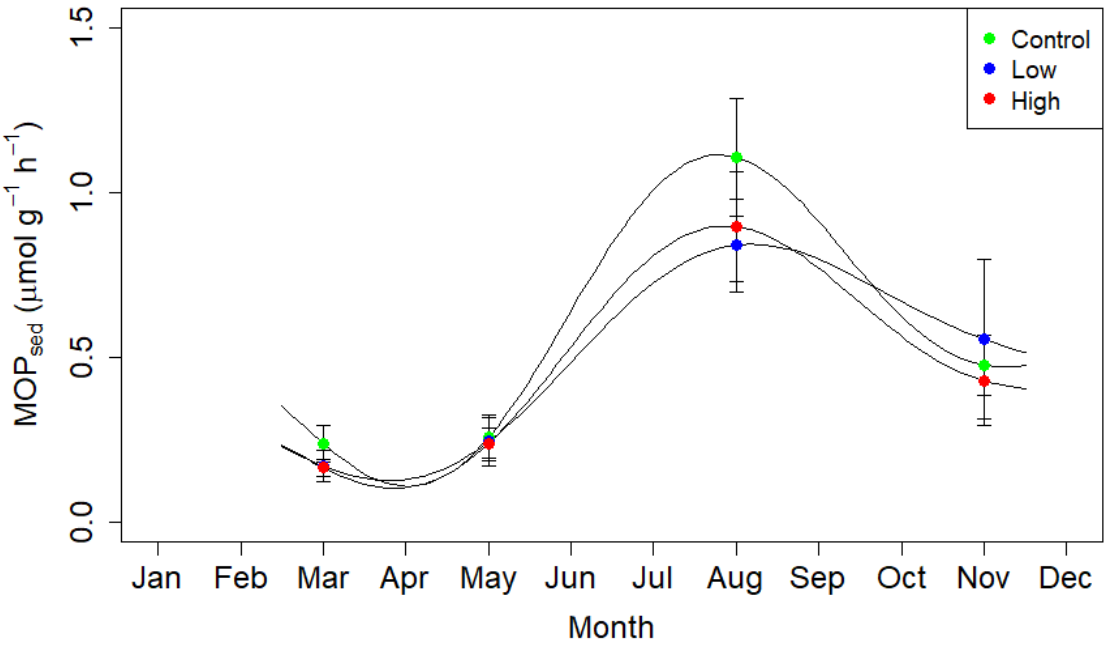

Figure 2.6: Seasonal variation in methane oxidation potentials of the surface sediment.
Mean (n = 8, ± 95% CI) methane oxidation potentials of the surface sediment for each crayfish density treatment across the year: red is high density, blue is low density, and green is control. Solid lines show fit of sinusoidal seasonal model for each treatment.

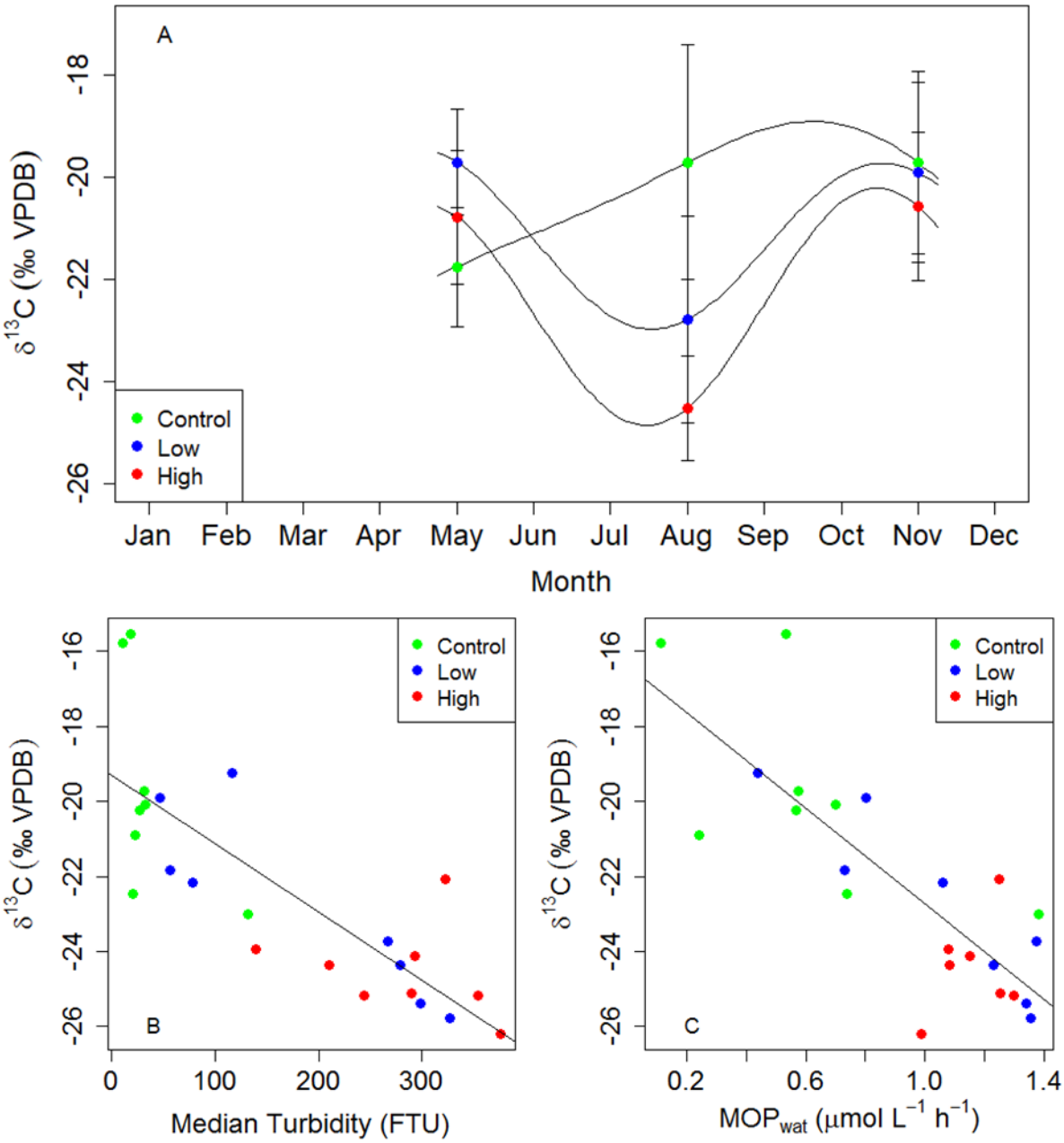

Figure 2.7: Seasonal variation in and factors influencing zooplankton $\delta^{13}C$ values in the mesocosms.
A) Mean (n = 8, ± 95% CI) $\delta^{13}C$ values of the zooplankton for each treatment across the year. Red is high density, blue is low density and green is control treatment. Solid lines show fit of 2 parameter sinusoidal seasonal model for each treatment. B) Linear relationship between bioturbation activity, measured as turbidity, and $\delta^{13}C$ of the zooplankton in the summer experiment (n = 24, R^2 = 0.63, y = -0.02x - 19.3). C) Linear relationship between methane oxidation potential of the water column and zooplankton $\delta^{13}C$ (n = 24, R^2 = 0.67, y = -6.4x - 16.4).

2.3.7 Zooplankton community structure

The zooplankton community structure displayed a distinct seasonal pattern (Figure 2.8). In the winter and autumn when bioturbation was low, there was no significant effect of crayfish density on zooplankton community structure (PERMANOVA; winter: $F_{2,21}$ = 0.041, p = 0.91; autumn: $F_{2,21}$ = 1.93, p = 0.10) . By contrast in the spring and summer when bioturbation was higher, the presence of crayfish was associated with a significant shift in the zooplankton community (PERMANOVA; spring: $F_{2,21}$ = 4.86, p <0.001; summer: $F_{2,21}$ = 4.97, p <0.001). In particular, total numbers of zooplankton increased dramatically (High density mean: 663 ± 64 indivs L^{-1}; control mean: 399 ± 49 indivs L^{-1}); Figure 2.9), with larger Daphniid species *D. pulex* and *D. magna* becoming dominant in the spring and summer, respectively. Additionally, smaller and/or rarer species that typically prefer vegetated habitats were almost completely excluded from crayfish mesocosms in both seasons, probably due to a large reduction in macrophyte coverage as a result of crayfish activity.

2.4 Discussion

This study has shown that while *P. clarkii* is already well known to shape ecological communities and its physical environment, it also has the potential to indirectly impact upon and fundamentally alter seasonal biogeochemical cycling and oxygen and methane dynamics.

The observed relationship between turbidity and crayfish density indicates that *P. clarkii* is responsible for suspension of fine sediment in the experimental mesocosms, thus agreeing with other studies that have identified *P. clarkii* as a major driver of sediment resuspension (Angeler *et al.*, 2001; Rodríguez *et al.*, 2003). Interestingly, this relationship appeared to be mostly linear at the population level in both spring and summer, indicating that agonistic behaviour did not increase with population density as expected, or if it did, its effects on bioturbation intensity were short-lived. At the individual mesocosm level however, there was wide variation in turbidity between mesocosms with the same number of crayfish. This non-linear variation suggests that there was considerable variety in bioturbation intensity between individual crayfish, which is supported by the fact that this species displays similar individual variation in ranging behaviour (Barbaresi *et al.*, 2004a). In the context of the mesocosm experiments this variety is useful since it creates a wide gradient of turbidity for the characterisation other bioturbation impacts, but in a natural system with a large connected population such individual

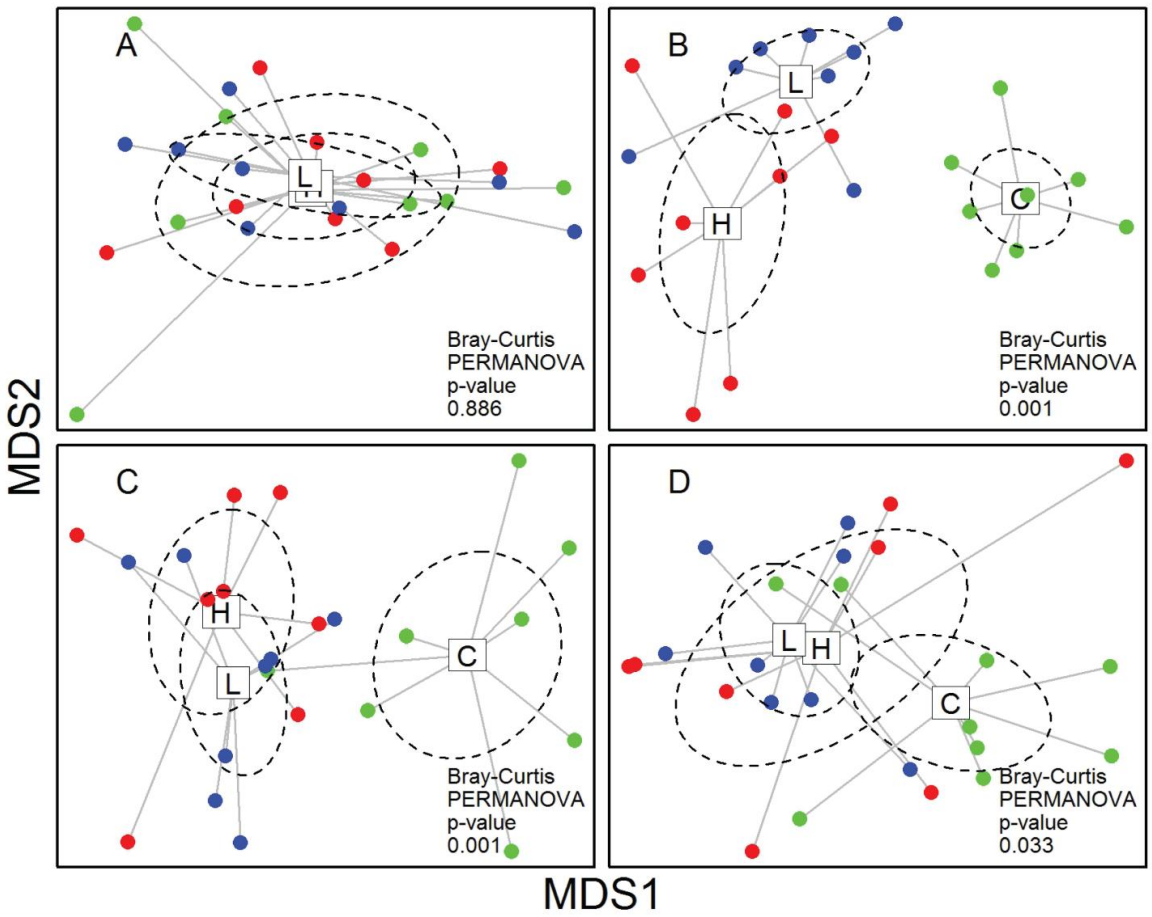

Figure 2.8: Ordination plots of zooplankton community structure in contrasting seasons.
Data shown are from the A) winter, B) spring, C) summer and D) autumn experiments. Centroid labels C, L and H represent control, low and high density crayfish treatments, respectively. Ellipses represent 95% confidence areas of the centroid. Axes are dimensionless with overlap of ellipses indicating similarity of community composition.

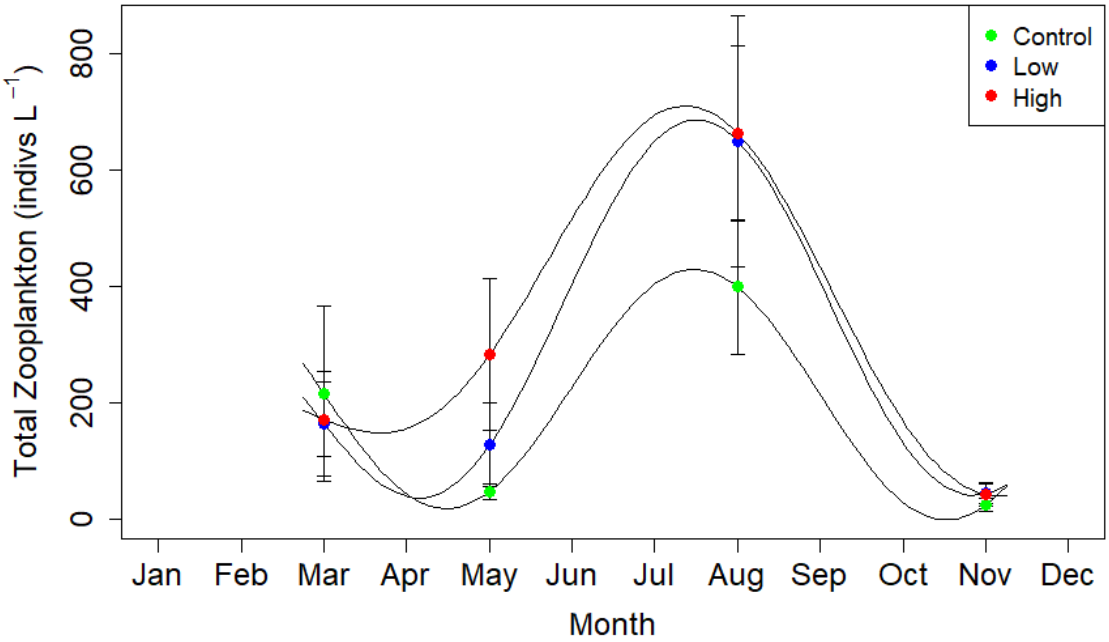

Figure 2.9: Seasonal variation in mesocosm total zooplankton populations.
Mean (n = 8, ± 95% CI) total zooplankton for each crayfish density treatment across the year: red is high density, blue is low density, and green is control. Solid lines show fit of sinusoidal seasonal model for each treatment.

effects would average out into a population level effect. *P. clarkii* bioturbation is strongly seasonal, such that it has a strong positive effect on turbidity in the spring and summer but no effect during autumn or winter. This large seasonal fluctuation is likely due to the fact *P. clarkii* is sub-tropical in origin and is therefore poorly adapted for colder temperatures, leading to much reduced activity during the autumn and winter. These results closely conform with a study by Gherardi et al. (2000) that found *P. clarkii* in an Italian wetland to be most active during the spring and summer months. The seasonal nature of *P. clarkii* bioturbation in a temperate climate is important since it means that its knock-on impacts will follow the same annual cycle.

The very strong relationships between chlorophyll *a*, NH_3 and turbidity in the summer supports the findings of Angeler et al (2001) and Yamamoto (2010) that bioturbation can be an important factor in the release of sediment bound nutrients and the formation of algal blooms; although, unlike Angeler et al (2001), this study found that N rather than P concentrations increased in response to bioturbation. The role of N in the limitation of freshwater phytoplankton has been downplayed in the past (Schindler, 1977), but more recent publications have identified that co-limitation by N and P is probably more widespread than by P alone (Guildford & Hecky, 2000; Maberly *et al.*, 2002; Elser *et al.*, 2007). Therefore, it seems likely that in this study, the increased release of N from the sediment due to bioturbation did contribute to the elevated algal biomass, in addition to the increased recruitment of previously sediment dwelling algae as suggested by Yamamoto (2010).

Algal blooms are often connected with the generation of oxygen deficits in bottom-water since their rapid turnover leads to the stimulation of microbial metabolism through elevated decomposition (Paerl and Otten 2013). In addition to this, we hypothesised that the increased exposure of re-suspended organic matter to oxygen, especially in the upper water column away from any bloom associated hypoxia, would further increase decomposition in more turbid mesocosms and so drive further oxygen depletion across the entire water column during periods with high bioturbation. The results of this study strongly support this idea since the mean daily dissolved oxygen concentrations just 10 cm below the water surface were strongly negatively related to turbidity during both the spring and summer. Furthermore, the fact that in the summer the daily maximum oxygen concentration declined linearly from ≈700 $\mu molL^{-1}$ in the least turbid mesocosms to ≈300 $\mu molL^{-1}$ in the most turbid indicates that not only was oxygen depletion present throughout the water column, but it also persisted through the daylight hours when photosynthetic production would have been very high due to the high algal biomass. Therefore, we can conclude that the biological oxygen demand of the water column must also

have been strongly related to bioturbation. This type of persistent seasonal oxygen depletion will probably make short-term hypoxia or anoxia much more likely during warmer months which can have serious ecological and/or economic impacts (Vanderploeg et al. 2009).

The increase in conductivity of the water in response to bioturbation indicates that this activity significantly changed the dissolved ionic content and hence the chemistry of the water. Given that water chemistry in addition to turbidity and chlorophyll are all known to affect zooplankton community composition (Cottenie et al. 2001) it was therefore expected that a shift in community composition would occur. This was confirmed by the fact that during the periods of low bioturbation in autumn and winter, when there was little variation in these factors, there was no consistent variation in community composition. Conversely, in spring and summer when turbidity, chlorophyll and conductivity all varied consistently between the treatments there was a marked difference in the mesocosms experiencing bioturbation compared to the control mesocosms. The shift in composition was mainly characterised by increased total numbers of zooplankton and dominance by large *Daphnia* species in bioturbated mesocosms; however, the overlap of the two crayfish treatments in both experiments shows that beyond a certain point additional turbidity, chlorophyll and dissolved ions did not continue to alter community structure. The indirect impact of crayfish bioturbation on the zooplankton community is potentially significant since zooplankton are a major pathway for the transfer of energy from primary producers to higher order consumers, therefore any change in their relative abundances could cascade upwards through the food-web. For example, zooplanktivorous fish are primarily visually-limited predators (particularly in turbid water) and so a shift towards dominance by a larger species such as *D. magna* may increase fish abundance at the expense of gape-limited invertebrate predators (Greene, 1983).

Previous investigations into the effect of bioturbation on methane dynamics have primarily focused on small bodied invertebrates such as chironomid larvae which alter the fine structure of the sediment thereby increasing oxygen penetration and thus promoting sediment methane oxidation (Kajan & Frenzel, 1999). However, this study found that alteration of the coarse sediment structure by crayfish bioturbation did not replicate this effect, and may even have had the opposite effect in the summer experiment when sediment methanotrophy was reduced under bioturbation, potentially due to low oxygen concentrations. In addition, this study found a dramatic and previously unexpected stimulation of pelagic methanotrophy associated with high levels of bioturbation in the spring and summer. This was likely due to a higher density of pelagic methane oxidising bacteria (MOB) due to large numbers being carried up into the water column

along with the sediment particles with which they are usually associated. This is likely to have made chemosynthetically derived energy much more freely available to the pelagic food web through the action of filter feeders such as zooplankton.

The zooplankton $\delta^{13}C$ values show a strong negative relationship with bioturbation activity. One possible reason for this is that zooplankton in more turbid waters will inevitably ingest and retain in their guts larger quantities of sediment, which can be somewhat more depleted in ^{13}C, thereby causing the zooplankton to appear more depleted in ^{13}C than they actually are (Feuchtmayr & Grey, 2003). However, this possibility is made less likely by the fact that the zooplankton were left in clean water overnight prior to being fixed to encourage gut clearance, so the amount of sediment in their guts was likely to be very low. In addition, even if gut clearance was not completely effective, the fact that the sediment $\delta^{13}C$ (-24.4‰ ± 0.18 SEM) falls within the range of measured zooplankton $\delta^{13}C$ values (-26.2 to -15.5‰) rather than beyond it, suggests that increased sediment ingestion cannot be the sole cause of ^{13}C depletion. Alternative potential causes of ^{13}C depletion include: A) increased ingestion of very low $\delta^{13}C$ MOB in the more turbid mesocosms (e.g. Grey, Kelly & Jones, 2004; Kankaala *et al.*, 2010); or B) ingestion of unusually low $\delta^{13}C$ phytoplankton (e.g. Lennon *et al.*, 2006). Scenario A would cause ^{13}C depletion because methane derived carbon typically has a $\delta^{13}C$ of around −80 to −60‰ (Jones & Grey, 2011), and so even a slight increase in ingestion of MOB could result in the observed depletion in $\delta^{13}C$ values. Scenario B could occur if the phytoplankton incorporates substantial amounts of heterotrophically respired CO_2 from low $\delta^{13}C$ organic matter rather than atmospheric CO_2. This is not especially uncommon and $\delta^{13}C$ values for lake phytoplankton of < −30‰ have been reported (Grey *et al.*, 2000; Vuorio *et al.*, 2006). These two scenarios are not mutually exclusive and the relative importance of each is impossible to elucidate without the use of additional tracers such as other stable isotopes (Taipale *et al.*, 2007) or analysis of phospholipid fatty acids which are diagnostic for MOB (Taipale *et al.*, 2009). However, support for scenario A is provided by the fact that in the summer, when bioturbation activity was highest, the zooplankton $\delta^{13}C$ was strongly negatively correlated with water column methanotrophy, suggesting that there may be a causal link. Our results therefore provide some support for previous studies which have found methane derived carbon to be supporting biomass at the highest trophic levels (Ravinet *et al.*, 2010; Sanseverino *et al.*, 2012). This alternative energy flow through the food web could fundamentally alter ecosystem functioning in crayfish invaded systems and, with the ever increasing spread of invasive crayfish globally, represents an important advance in our understanding of how such systems work and so further work on this subject is recommended.

Since this study was conducted as a mesocosm experiment, it is necessary to consider the relevance of the results to more natural systems. The mesocosms were designed to simulate shallow ponds and lakes and shallow littoral areas with extremely low flow within larger water bodies since these are the areas most frequently occupied by invasive crayfish (Ruokonen et al. 2012). The consistency of the results obtained with those in the published literature for more natural systems, notably with regards turbidity, chlorophyll, nutrients and conductivity (Angeler *et al.*, 2001; Rodríguez *et al.*, 2003; Hänfling *et al.*, 2011), suggests that the mesocosms were relatively successful representations of natural systems. The applicability of the results to other crayfish species will likely depend on the extent of their bioturbation potential, which is unclear for many species, although the signal crayfish, *Pacifastacus leniusculus*, is known to cause significant bioturbation in a similar way to *P. clarkii* and so it would be reasonable to expect similar effects from this species.

Chapter 3: Comparison of bioturbation impacts with other crayfish species

3.1 Introduction

In chapter 2 it was shown that *Procambarus clarkii* bioturbation has the potential to impact upon numerous ecosystem properties and processes and that these impacts vary according to a seasonal cycle. For that detailed year-round study *P. clarkii* was chosen since it is a widespread species that is well known for its high bioturbation activity (Angeler *et al.*, 2001; Rodríguez *et al.*, 2003; Barbaresi *et al.*, 2004b). However, globally there are over 20 other species of crayfish that have been introduced outside their native ranges, with many now well established at multiple locations on two or more continents (Rodríguez & Suárez, 2001; Hänfling *et al.*, 2011). Indeed, in the UK alone there are currently at least seven established non-native crayfish species (Kouba *et al.*, 2014). Furthermore, every crayfish species will inevitably have its own unique ecology, physiology and behaviour patterns, which will determine the nature and extent of its impacts on the systems it invades. Consequently, it is important to understand how the outcomes of the *P. clarkii* experiment presented in Chapter 2 compare to other species, particularly those that are also widespread, in order to better understand the global impact of crayfish bioturbation in general.

The Signal crayfish, *Pacifastacus leniusculus*, which originates from north-western North America, is one such species that has a large invasive range, being well established across much of western, central and northern Europe and Japan (Rodríguez & Suárez, 2001; Kouba *et al.*, 2014). *P. leniusculus*, is commonly regarded as an invasive pest outside its native range due to its ability to dramatically alter recipient community structure through direct predation (Nystrom *et al.*, 1999) and its ability to impact native crayfish species through transmission of the Crayfish Plague (Alderman *et al.*, 1990). With regards its effect on the sediment, *P. leniusculus* was originally thought to be a non-burrowing species (Hogger, 1988), although it has since been demonstrated to excavate burrows and pits outside of its native range (Guan, 1994). Burrowing activity has been recorded to be greatest amongst individuals with carapace lengths <50mm, with larger individuals more commonly digging pits rather than burrows (Guan, 1994). These activities have been documented to actively disturb the sediment, increasing water turbidity and altering sediment transport (Johnson *et al.*, 2010; Harvey *et al.*, 2011, 2014).

The Turkish Crayfish, *Astacus Leptodactylus*, which is native to Eastern Europe and the Middle-East, is another species that has large invasive range, being established throughout much of Central and Western Europe (Kouba *et al.*, 2014). Despite its wide distribution and unlike *P. leniusculus*, there are relatively few reports indicating that *A. leptodactylus* causes significant environmental degradation, although the few studies there are indicate that this species is capable of reaching high densities, of impacting native benthic invertebrate populations and displacing native crayfish species (Holdich, 1999; Holdich *et al.*, 1999; Jackson *et al.*, 2014). There are however no reports in the literature of burrowing or other bioturbation activity by *A. leptodactylus*.

Given the wide distribution of *P. leniusculus* and *A. leptodactylus*, it is considered that these two species provide a good platform to assess how the results from the *P. clarkii* experiment compare to other species and therefore to assess the likely impact of crayfish bioturbation on a global scale. On the basis of the available literature for both *P. leniusculus* and *A. leptodactylus* and the results of the *P. clarkii* experiments presented in Chapter 2 it is possible to hypothesise in general terms the potential bioturbation impacts of these two species as follows:

1. *P. leniusculus* is an active burrower and so would be expected to cause significant bioturbation, including sediment resuspension, although given that burrowing activity declines with size and that it was considered a non-burrowing species in its native range, the intensity of bioturbation may well be lower than that of *P. clarkii*.
2. *A. leptodactylus* is not reported to burrow and so would not be expected to cause as much bioturbation as *P. clarkii*, although some bioturbation would still be expected due to its large size.
3. Bioturbation activity by both *P. leniusculus* and *A. leptodactylus* may be maintained throughout more of the year than *P. clarkii* since they originate from temperate rather than sub-tropical climatic zones and are therefore more likely to be adapted to colder temperatures.
4. Bioturbation by either species would result in the same general impacts as observed for *P. clarkii* on dissolved oxygen, methane dynamics, algal biomass and zooplankton community structure, although the extent of these impacts will likely be lower, as a result of the expected lower bioturbation activity of these species.

The study presented in this chapter was designed to test the above hypotheses through repetition of the experimental mesocosm experiment presented in Chapter 2 with *P. leniusculus* and *A. leptodactylus.*

3.2 Methods

3.2.1 Experimental design

The experimental mesocosms were setup exactly as described in section 2.2.1 in order to maximize comparability between experiments. The only changes were to the crayfish treatment, with *P. leniusculus* and *A. leptodactylus* being used instead of *P. clarkii* and the use of 25 mesocosms rather than 24 to maintain a balanced design. There was insufficient time to conduct a full seasonal study, as was undertaken for *P. clarkii*, and so it was intended to focus on spring and summer, the likely periods of highest activity, in order to identify the maximum impacts of each species. However, whilst the spring experiment was conducted successfully, the summer experiment failed due to a likely disease outbreak that resulted in very high mortality of the crayfish, especially *A. leptodactylus*, within both the experimental mesocosms and the indoor storage tanks, such that the crayfish density treatments could not be maintained. Consequently, the summer experiment was abandoned and a new experiment was setup with new crayfish stocks in the autumn. As a result, these experiments miss out the likely period of greatest activity and therefore impact. Nevertheless, the spring and autumn experiments are still considered sufficient to identify the major impacts of bioturbation by these species, if not the maximum magnitude of these impacts. The experiments were conducted in the same months as the spring and autumn experiments presented in chapter 2 (May and November) and temperature measurements were taken to ensure comparability with the previous experiments.

At the start of each experiment one of five crayfish density treatments was applied to each mesocosm: zero crayfish (controls, n = 5); two *P. leniusculus* (low density, 4 individuals m^{-2}, n = 5); four *P. leniusculus* (high density, 8 individuals m^{-2}, n = 5); two *A. leptodactylus* (low density, 4 individuals m^{-2}, n = 5) and four *A. leptodactylus* (high density, 8 individuals m^{-2}, n = 5). These densities were chosen in order to match those used for the *P. clarkii* study in order to maintain comparability between the experiments. These densities were originally chosen for the previous study since they fell within the range of *P. clarkii* densities that have been reported in the field (Momot *et al.*, 1978; Gherardi & Lazzara, 2006; Chucholl, 2013); however they are also within densities at which *P. leniusculus* has been reported (Guan & Roy Wiles, 1996). Quantitative estimates of *A. leptodactylus* densities are lacking in the literature, however their catch per unit effort is typically high and so it is plausible that these densities are representative for this species as well. These numbers also allowed a constant sex ratio of 1:1 to be maintained across both treatments, thereby reducing the behavioural variation between the mesocosms. All the crayfish used were adults, had intact and equal chelae and carapace lengths of 40-70mm. The

mesocosms were regularly checked for dead crayfish, which were immediately removed and replaced. Each experiment was run for six weeks, after which all data was collected.

3.2.2 Measurements

Measurements of turbidity, dissolved oxygen and chlorophyll *a* concentrations, methane dynamics and zooplankton community structure were taken for each mesocosm as described in section 2.2. The 25 mesocosms were divided into five geographic blocks of five mesocosms each, with one of each crayfish species and density treatment and one control treatment per block. Measurements of turbidity and oxygen were taken simultaneously over 24 hours for mesocosms within the same block, with all five blocks being measured over five consecutive days. Measurements of nutrient concentration and conductivity were not taken in these experiments since these are previously documented effects of sediment resuspension and were primarily used in the *P. clarkii* experiment to check whether the mesocosm setup was capable of replicating effects observed in natural systems and therefore whether they were effective simulations of those systems.

3.2.3 Data analysis

Since data were only collected for two seasons (spring and autumn) the three parameter sinusoidal seasonal model (described in section 2.2.10) could not be employed since there were insufficient degrees of freedom available. Consequently the data were analysed with linear mixed effects models with simple fixed effects for season and crayfish density and random effects for geographic block, to control for variations due to location of the mesocosms.

For analysis of turbidity and dissolved oxygen, measures of central tendency were taken for each mesocosm for both datasets; the median was used for turbidity since some of the high density treatments were skewed by occasional spikes in turbidity, whilst the mean was used for dissolved oxygen. All relationships within each experimental run were assessed with linear models using type II Sums of Squares. The zooplankton communities were compared between treatments with MDS ordination techniques and analysed by PERMANOVA. All zooplankton scores were transformed with square roots to prevent the marginalisation of rare species. All statistical analyses were done using R statistical software (R Development Core Team 2017).

3.3 Results

3.3.1 Turbidity

Turbidity had a significant relationship with season for both *P. leniusculus* ($F_{1,22}$ = 48.1, p < 0.001) and *A. leptodactylus* ($F_{1,22}$ = 42.9, p < 0.001), with turbidities being higher in the spring than in the autumn (Figure 3.1). Turbidity also had a positive relationship with crayfish density for both species (Signal: $F_{2,22}$ = 7.76, p < 0.01, Turkish: $F_{2,22}$ = 14.7, p < 0.001). The turbidity difference between densities was slightly larger during the spring, however the lack of a significant interaction term for either species indicates that bioturbation activity was not greatly enhanced during the spring. In addition, although both species were undertaking bioturbation activity, the mean increase in turbidity relative to the controls during the spring was much smaller for both species (Signal: +20.0 FTU, Turkish: +24.6 FTU) than for *P. clarkii* (+98.6 FTU), indicating that they are less effective biotubators.

3.3.2 Dissolved Oxygen

Dissolved oxygen concentrations had a significant relationship with season for both *P. leniusculus* ($F_{1,22}$ = 292, p < 0.001) and *A. leptodactylus* ($F_{1,22}$ = 320, p < 0.001), with concentrations being lower in the spring than in the autumn. In part, the lower absolute concentrations in the spring are due to the fact that oxygen becomes less soluble in water with increasing temperature, however, the same relationship can be observed in the percentage oxygen saturation, which controls for differences in oxygen solubility (Figure 3.2). Oxygen concentrations also had a significant negative relationship with crayfish density for both species (Signal: $F_{2,20}$ = 7.79, p < 0.01, Turkish: $F_{2,19}$ = 7.69, p < 0.001). The interaction between season and crayfish density was just non-significant at the alpha = 0.05 level for both species, however the models fitted the data better when the interaction term was included, indicating that seasonal fluctuations in mean dissolved oxygen concentrations were greater in mesocosms with higher bioturbation activity; however, this conclusion cannot be confirmed without more data or data from a season when the crayfish were even more active.

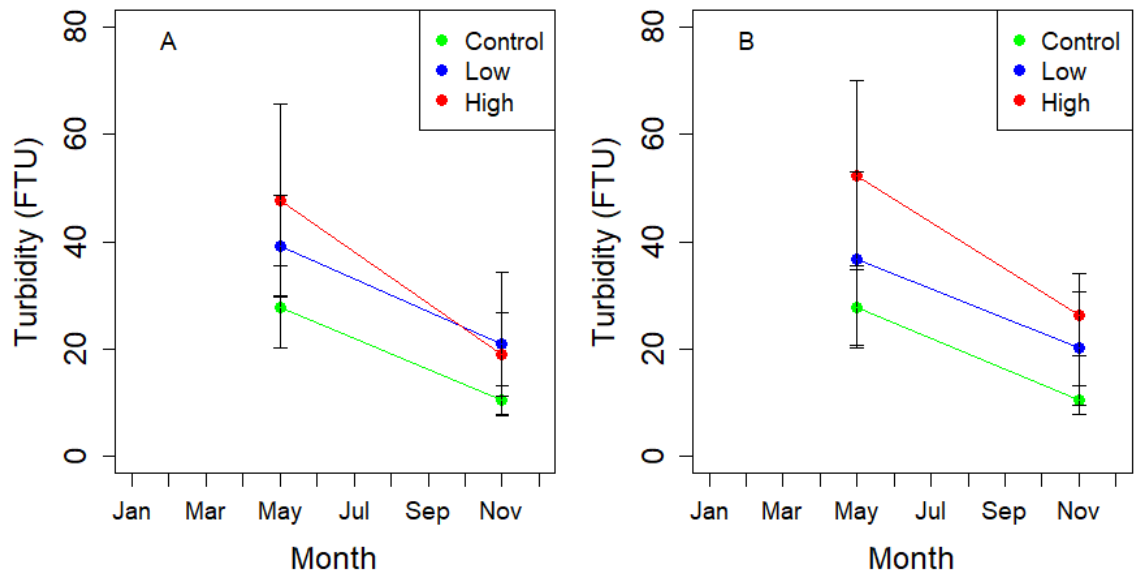

Figure 3.1: Median turbidity in mesocosms in the spring and autumn.
A) *P. leniusculus* and B) *A. leptodactylus*. Red circles represent high density treatment (n=5), blue circles are low density treatment (n = 5) and green circles are the control treatment (n=5). Error bars show 95% confidence intervals.

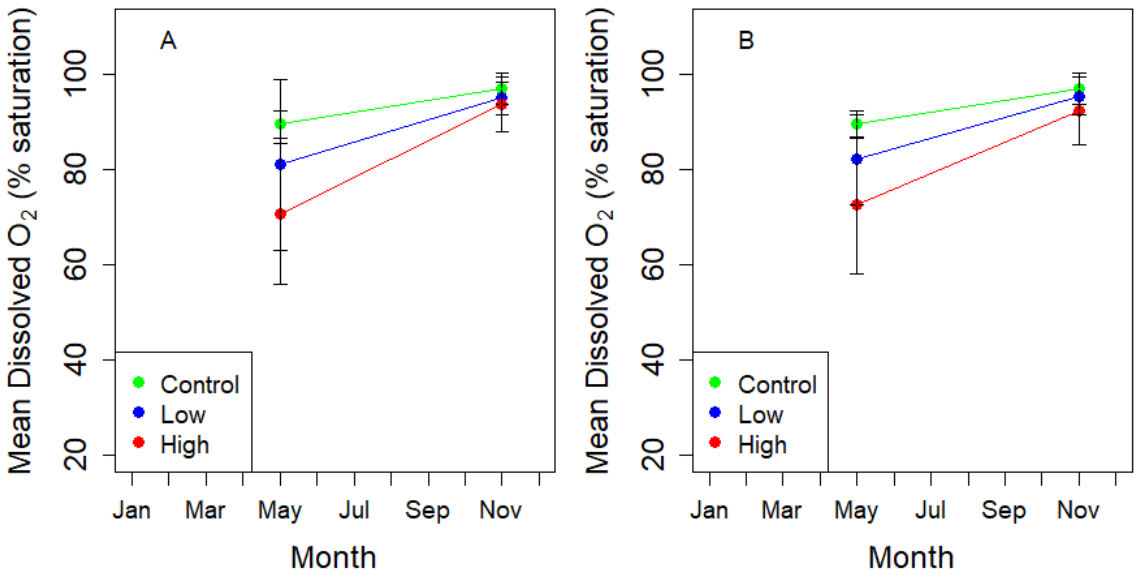

Figure 3.2: Mean (n = 5, ± 95% CI) dissolved oxygen saturation in mesocosms in the spring and autumn.
A) *P. leniusculus* and B) *A. leptodactylus*. Red circles are high density treatment, blue circles are low density treatment and green circles are the control treatment

3.3.3 Chlorophyll a

Chlorophyll *a* concentrations had a significant relationship with season for both *P. leniusculus* ($F_{1,21}$ = 6.74, p < 0.05) and *A. leptodactylus* ($F_{1,22}$ = 12.7, p < 0.01), with concentrations being slightly higher in the autumn (Figure 3.3). However, the actual concentrations recorded were very low in both seasons (<10 µg L^{-1}) and so the inter-season difference is probably not of much ecological importance. Chlorophyll *a* concentrations also had a positive relationship with crayfish density for both species (Signal: $F_{2,21}$ = 3.74, p < 0.05, Turkish: $F_{2,22}$ = 16.2, p < 0.001), with algal biomass being highest in the most turbid mesocosms for *P. leniusculus* in the spring ($F_{1,13}$ = 7.77, p < 0.05).

3.3.4 Methanogenesis, dissolved methane, and methane oxidation

Methanogenic potential (MGP) showed a seasonal relationship with the highest rates of methanogenesis being observed during the autumn experiment. Crayfish density was found to have no significant relationship with methanogenesis. However, the data were hugely variable both within and between experiments and so it is unlikely that any meaningful conclusions can be drawn from these observations.

Dissolved methane concentrations were oversaturated relative to atmospheric equilibration (3.2 nmol L^{-1} at 10°C) with a mean of approximately 1.3 µmol L^{-1} in both spring and autumn for both species. Neither crayfish density nor season was found to have any explanatory power for methane concentrations for either species (Figure 3.4).

Methane oxidation potential of the water column (MOP_{wat}) was strongly seasonal (Figure 3.5), with MOP_{wat} being higher in the spring than in the autumn for both species (Signal: $F_{1,22}$ = 1174, p < 0.001, Turkish: $F_{1,22}$ = 1342, p < 0.001). No relationships were found between either crayfish density or turbidity and MOP_{wat} for either species in either season.

Methane oxidation potential of the surface sediment was strongly seasonal (Figure 3.6), with MOP_{sed} being higher in the spring than in the autumn for both species (Signal: $F_{1,22}$ = 76.5, p < 0.001, Turkish: $F_{1,22}$ = 110, p < 0.001). Neither crayfish density nor turbidity was found to have a significant relationship with MOP_{sed} for either species.

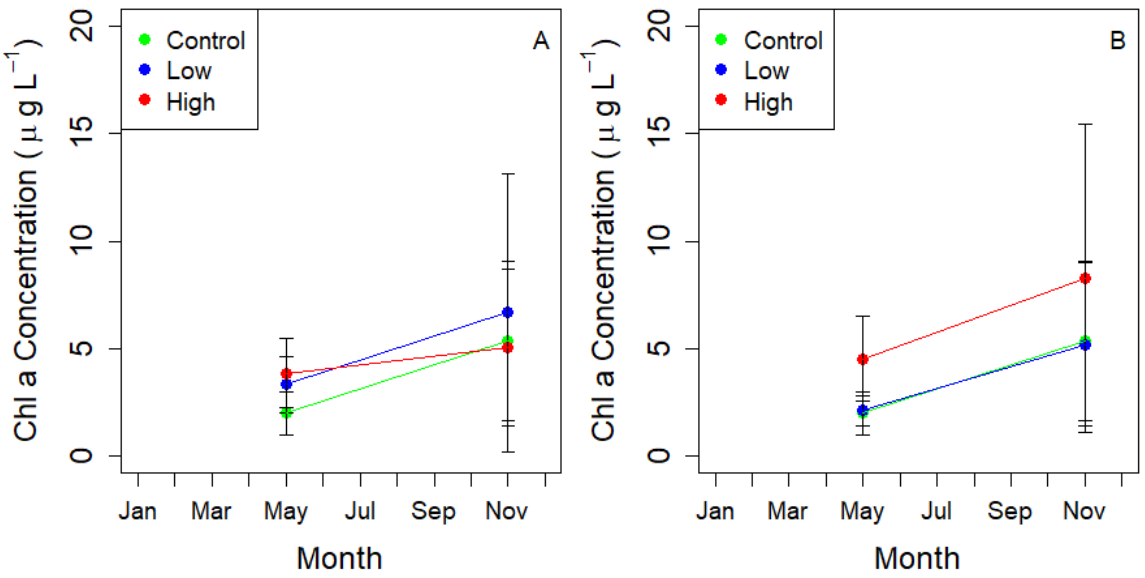

Figure 3.3: Mean (n = 5, ± 95% CI) chlorophyll *a* concentrations for each treatment in the spring and autumn.
A) *P. leniusculus* and B) *A. leptodactylus*. Red is high density treatment, blue is low density and green is control treatment.

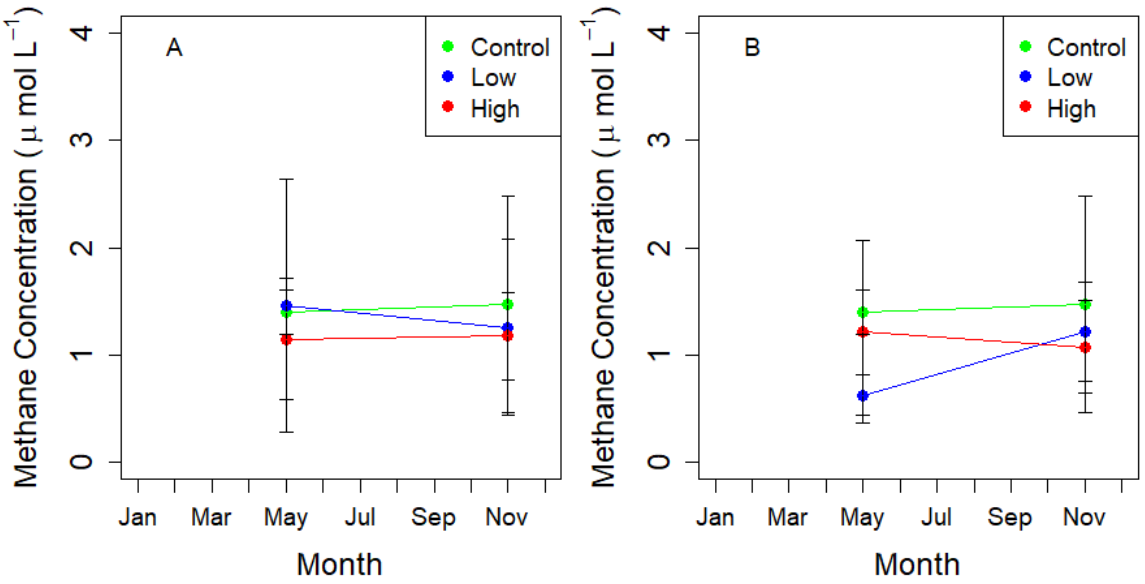

Figure 3.4: Mean (n = 5, ± 95% CI) dissolved methane concentrations for each treatment in the spring and autumn.
A) *P. leniusculus* and B) *A. leptodactylus*. Red is high density treatment, blue is low density and green is control treatment.

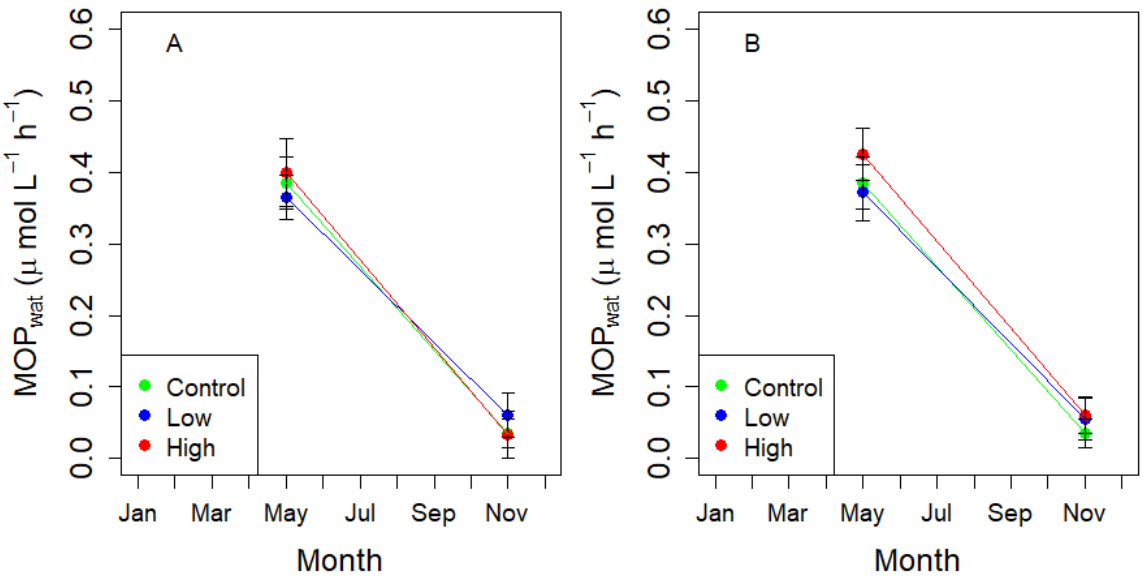

Figure 3.5: Mean (n = 8, ± 95% CI) methane oxidation potentials of the water column for each treatment in the spring and autumn.
A) *P. leniusculus* and B) *A. leptodactylus*. Red is high density treatment, blue is low density and green is control treatment.

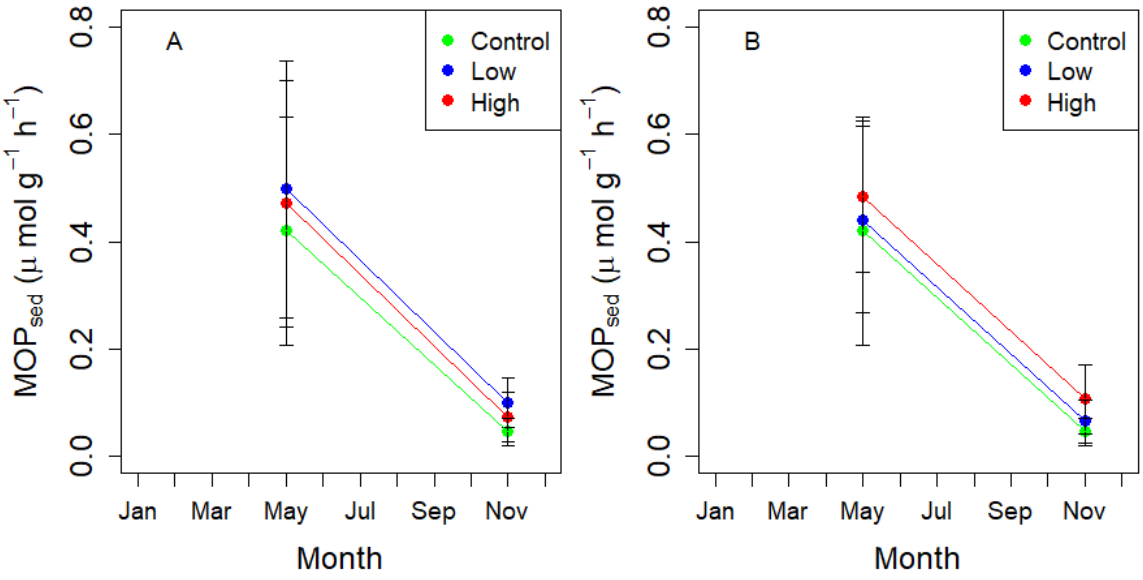

Figure 3.6: Mean (n = 8, ± 95% CI) methane oxidation potentials of the surface sediment for each treatment in the spring and autumn.
A) *P. leniusculus* and B) *A. leptodactylus*. Red is high density treatment, blue is low density and green is control treatment.

3.3.5 Zooplankton community structure

The zooplankton community structure varied significantly with crayfish density for both species in both seasons (Figure 3.7). For *P. leniusculus*, the zooplankton communities in the 4 crayfish mesocosms were conspicuously different from the controls in both seasons, with the 2 crayfish mesocosms being highly variable in the spring and similar to the controls in the autumn. For *A. leptodactylus*, the 4 crayfish mesocosms were again different from the controls in both seasons, with the 2 crayfish mesocosms being similar to the high density treatment in the spring and intermediate between the 4 crayfish and control treatments in the autumn. In all experiments, the main difference between the 4 crayfish and control treatments was an elevated abundance of large *Daphnia* species relative to smaller copepod species.

3.4 Discussion

This study has shown that *P. leniusculus* and *A. leptodactylus* produce similar levels of bioturbation as each other in the spring and autumn and that this bioturbation has the potential for knock-on impacts upon oxygen, methane and community dynamics. Both species are less effective bioturbators than *P. clarkii* and so the magnitude of all impacts is likely to be smaller over the full annual cycle. However, the exact nature of the complete annual cycle of impacts for these species cannot be fully determined without either more experiments at other times of year or a more complete understanding of how bioturbation activity varies for each species over the course of the year.

The observed positive relationships between turbidity and crayfish density demonstrate that both *P. leniusculus* and *A. leptodactylus* are active bioturbators. However, the mean increase in turbidity relative to the controls during the spring was much smaller for both species (Signal: +20.0 FTU, Turkish: +24.6 FTU) than for *P. clarkii* (+98.6 FTU), indicating that they may be less effective biotubators. In contrast, in the autumn both species maintained a low level of bioturbation activity (Signal: +10.4 FTU, Turkish: +16.0 FTU) whilst *P. clarkii* did not produce any significant bioturbation. This suggests that whilst *P. leniusculus* and *A. leptodactylus* are less effective bioturbators, they do appear to be active over a wider range of the year and so any impacts of their bioturbation are likely to be less seasonal than for *P. clarkii*. This is supported by previous work done by Johnson *et al.* (2014), Bubb *et al.* (2002) and Gherardi *et al.* (2000) which found that *P. leniusculus* activity remained high well into the autumn / winter until temperatures

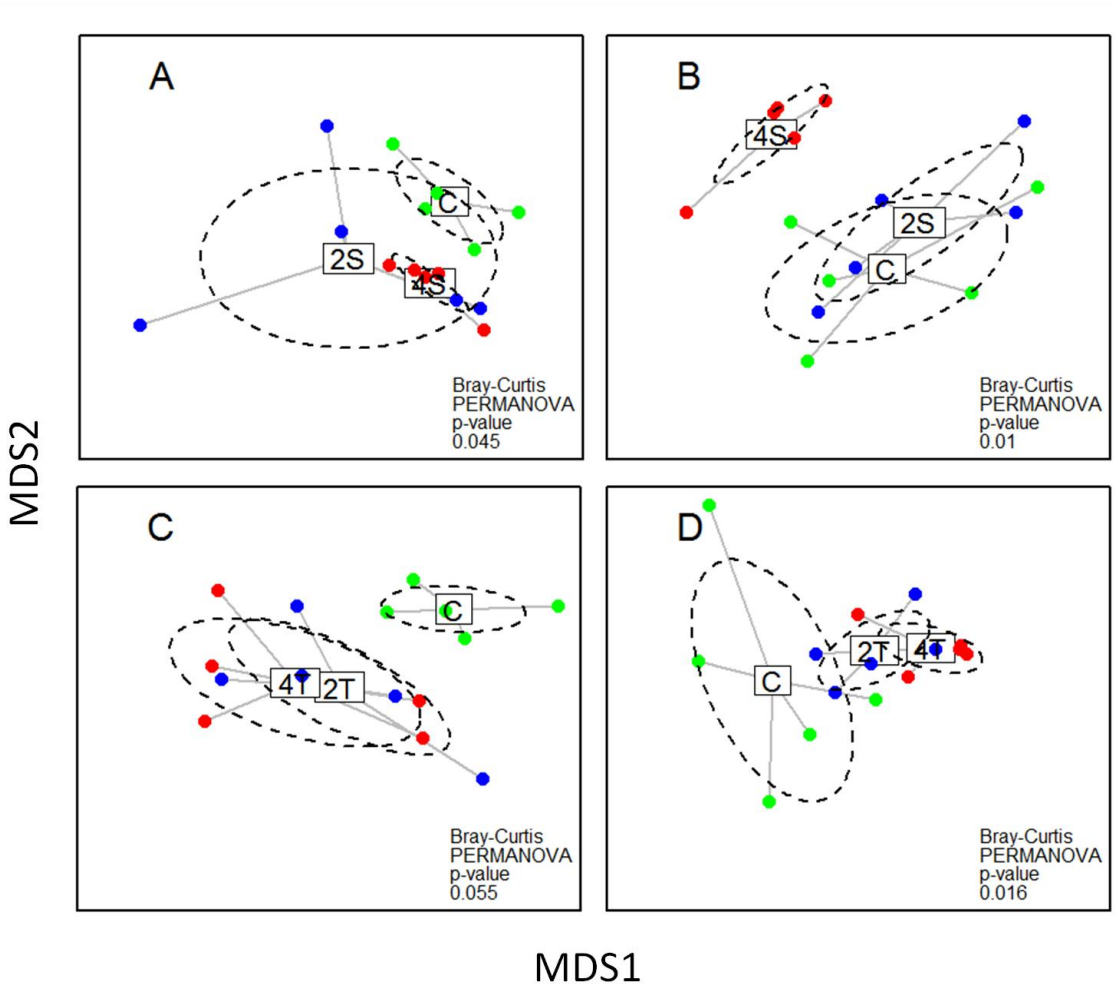

Figure 3.7: Ordination plots of zooplankton community structure in contrasting seasons.
Data shown are from the A) *P. leniusculus* spring, B) *P. leniusculus* autumn, C) *A. leptodactylus* spring and D) *A. leptodactylus* autumn experiments. Centroid labels C, 2S, 4S, 2T and 4T stand for control, 2 Signal, 4 Signal, 2 Turkish and 4 Turkish crayfish treatments respectively. Ellipses represent 95% confidence areas of the centroid. Axes are dimensionless with overlap of ellipses indicating similarity of community composition.

fell below 5°C , whilst *P. clarkii* activity declined dramatically in the autumn when temperatures fell below 15°C. The likely reason for these different patterns of seasonal activity is that the native ranges of *P. leniusculus* and *A. leptodactylus* occur in a temperate climatic zone, whilst *P. clarkii* originates from a subtropical climate and so it is likely that *P. leniusculus* and *A. leptodactylus* have evolved to cope with periods of cold weather whilst *P. clarkii* has not.

The comparatively low bioturbation produced by *P. leniusculus* in these experiments is perhaps somewhat unexpected since this species has been documented to be an active burrower and to produce significant sediment resuspension in previous studies (Guan, 1994; Johnson *et al.*, 2010; Harvey *et al.*, 2011, 2014). Furthermore, the similar turbidities produced by *P. leniusculus* and *A. leptodactylus* are equally unexpected since there are no reports in the literature of *A. leptodactylus* undertaking burrowing or other significant bioturbation activity. There are however, a number of possible explanations for the apparent discrepancies between these results and the published literature. First of all it is possible that the *A. leptodactylus* results are not atypical and that the absence of published literature on bioturbation by this species represents a lack of studies or reporting rather than indicating that this species is not an active bioturbator. Indeed, during these experiments and those presented in Chapter 4, *A. leptodactylus* was observed to undertake bioturbation activities, notably the digging of shallow pits and the pushing of its large claws through the sediment as it walked (in the manner of a snow plough), both activities which were observed to result in sediment resuspension.

With regards *P. leniusculus,* it is possible that the relatively large size of the individuals used (carapace length 40-70mm) resulted in lower than expected bioturbation intensity, since Guan (1994) reported that individuals with carapace lengths over 50mm undertook significantly less burrowing activity than smaller individuals. Consequently, whilst the sizes of crayfish were chosen for consistency between experiments, it is possible that this may have led to an underestimation of the species level bioturbation potential / impact for *P. leniusculus.* Alternatively, it is possible that the artificial nature of the mesocosms, particularly the lack of predators and earth banks resulted in lower bioturbation activity than would be expected in a more natural system. In particular, *P. leniusculus* burrows are typically recorded in earth banks and therefore their absence within this experimental design may have led to an underestimation of bioturbation activity by this species. Nevertheless, *P. clarkii* burrows are also typically found in earth banks and so whilst the turbidities produced in these experiments may not be truly indicative of those produced in a natural system, they should be sufficient to allow effective comparison of general bioturbation potential between these species.

Given that both *P. leniusculus* and *A. leptodactylus* did undertake some bioturbation activity and thus cause sediment resuspension we would expect to see a concomitant effect on dissolved oxygen concentrations due to accelerated rates of organic matter decomposition as was seen for *P. clarkii.* Indeed this was observed, with higher levels of bioturbation activity (measured either as crayfish density or turbidity) being associated with an oxygen deficit relative to the control mesocosms. The detected oxygen deficit for both *P. leniusculus* and *A. leptodactylus* high density treatments (-20%) was smaller than for *P. clarkii* (-31%) in the spring but larger in the autumn (-5% vs. 0%). These results fit well with the idea that the oxygen deficits are driven by increased decomposition of suspended organic matter since they closely mirror the patterns of turbidity observed for each species. Therefore, these results suggest that *P. leniusculus* and *A. leptodactylus* may have a lesser impact on dissolved oxygen concentrations than *P. clarkii* but what impact they have may be present over a greater part of the annual cycle.

Oxygen concentrations did not become hypoxic (<30% oxygen saturation) for either *P. leniusculus* or *A. leptodactylus*; however, it is possible that this effect would be additive with other drivers of oxygen depletion such as eutrophication or pollution and so could tip systems into hypoxia when co-occurring. It is likely that both species would produce larger oxygen deficits in the summer, as was seen with *P. clarkii*, since they may become more active and thus cause more sediment resuspension. In addition, higher temperatures during the summer would accelerate microbial metabolism and thereby potentially exacerbate the observed oxygen depletion even if sediment resuspension did not increase significantly. Alternatively, if bioturbation activity did not increase much beyond that observed in the spring, the elevated nutrient concentrations due to accelerated decomposition, as documented for *P. clarkii* and in the literature (Angeler *et al.*, 2001), would likely increase algal biomass and perhaps trigger algal blooms which are themselves known drivers of oxygen deficits (Paerl & Otten, 2013). The observed data for algal biomass offer some support to this idea since chlorophyll *a* concentrations were positively related to bioturbation activity for both species in the spring, however, given the low algal biomass in the spring it would be necessary to have data from the period of highest algal growth (i.e. the summer) to confirm this supposition.

Given that turbidity, dissolved oxygen and algal biomass were all related to bioturbation activity and all of these are known drivers of zooplankton community composition (Cottenie *et al.*, 2001) it was expected that a shift in the zooplankton community structure would be observed in response to bioturbation, as was seen for *P. clarkii*. This shift was indeed detected for both *P. leniusculus* and *A. leptodactylus*, with the communities in the high density mesocosms being

significantly different from those in the controls in both the spring and autumn, whilst the low density mesocosms were somewhat more variable between the two. The shift in composition was mainly characterised in all cases by an elevated abundance of large *Daphnia* species relative to smaller copepod species, with smaller and/or rarer species that typically prefer vegetated habitats being almost completely excluded from crayfish mesocosms in both seasons, probably due to a reduction in macrophyte coverage as a result of crayfish bioturbation activity. This shift in the zooplankton community could be important in a natural system since zooplankton are a major food resource for the pelagic food web and so any changes could cascade upwards through the system. An important feature of the data is that there were still strong differences between the zooplankton communities of the high density and control mesocosms in the autumn for both *P. leniusculus* and *A. leptodactylus*, unlike for *P. clarkii*. This gives further support to the idea that the impacts of these two species span a broader part of the annual cycle than those of *P. clarkii*.

Methane dynamics were less impacted by *P. leniusculus* and *A. leptodactylus* bioturbation than by *P. clarkii*. Methane oxidation in the water column was only found to vary with *A. leptodactylus* density, although this could not also be linked with turbidity and so it is not possible to say for certain whether this is the result of crayfish bioturbation. The likely reason for this small or non-existent impact is that the amount of sediment that these species put into suspension was much less than *P. clarkii* did and so any increase in the number of methane oxidising bacteria in the water column and hence the methane oxidation potential would also have been small. This is supported by the fact that all turbidities and methane oxidation potentials recorded in the spring for both *P. leniusculus* and *A. leptodactylus* fall within the ranges of the low density and control treatments in the comparable spring *P. clarkii* experiment, which were also not significantly different from each other. It therefore appears that at this sampling resolution it requires a larger increase in turbidity to have any detectable effect on water column methane oxidation potential.

Another difference found between this and the *P. clarkii* study is that the sediment surface methane oxidation potential appeared to be enhanced by bioturbation activity by both *P. leniusculus* and *A. leptodactylus* but was not affected by *P. clarkii*. A possible explanation for this is that crayfish bioturbation is increasing the oxygen penetration depth of the surface sediment thereby promoting methane oxidation, as has been documented for chironomid larvae and bethivorous fish (Kajan & Frenzel, 1999; Ritvo *et al.*, 2004). This may not have been detected in

the *P. clarkii* experiment since lower dissolved oxygen concentrations may have counteracted this effect.

Overall, *P. leniusculus* and *A. leptodactylus* are capable of producing significant bioturbation and impacting upon oxygen, methane and community dynamics. Both species are less effective bioturbators than *P. clarkii* in the spring but appear to remain active over a broader part of the year and so the magnitude of all impacts is likely to be smaller but more prolonged over the annual cycle. However, the exact nature of the complete annual cycle of impacts for these species cannot be fully determined without either more experiments at other times of year or a more complete understanding of how bioturbation activity varies for each species over the course of the year.

Chapter 4: The impact of temperature on crayfish bioturbation intensity and its use in extrapolation of mesocosm experimental results

4.1 Introduction

In chapters 2 and 3, it has been demonstrated that bioturbation by the invasive crayfish species *P. leniusculus*, *A. leptodactylus* and in particular *P. clarkii* has the potential to dramatically impact upon a wide range of ecosystem properties and processes. However, it is important to note that the overall significance of any biological process in the shaping of an ecological community is heavily dependent on the abiotic context in which that process occurs (Power *et al.*, 1988; Poff & Ward, 1989). In temperate freshwater ecosystems, which the mesocosms were designed to simulate, an abiotic factor of great importance is temperature since it varies seasonally and is a powerful driver of the rate of many biological processes. This is particularly the case for poikilothermic organisms such as fish or crayfish which are known to be more active and have higher feeding and growth rates at higher temperatures (Kerkut & Taylor, 1958; Seals *et al.*, 1997; Croll & Watts, 2004; Harlioğlu, 2009). Consequently, the extent of crayfish bioturbation and its impacts should vary with temperature and therefore seasonally. Indeed, a study by Fortino (2006) found that bioturbation by the crayfish species *Cambarus chasmodactylus* and *Orconectes cristavarius* and several fish species declined in accordance with reducing temperatures, to the extent that no bioturbation was detectable during the winter months. Similarly, another study by Canal *et al.* (2015) found that sediment disturbance by three different fish species was 2-3 times higher at 20°C than 10°C. Given the seasonal variation of temperature, it is therefore important to quantify exactly how crayfish bioturbation activity varies with temperature in order to fully understand its overall importance throughout the year for each species.

For *P. leniusculus* and *A. leptodactylus* there is particular need for this information since the outdoor mesocosm experiments presented in chapter 3 were only conducted in the spring and autumn and thus potentially missed the periods of highest and lowest crayfish bioturbation activity during the warmest and coldest parts of the year. For instance, the mean temperature during the spring experiment was only 14°C but both species have been found to be more active at 25°C than 15°C in a mesocosm environment (Harlioğlu, 2009) and so they may be capable of producing greater bioturbation at higher temperatures during the summer. In addition, given

the that both species evolved in a temperate climatic zone, it is possible that they are less affected by low temperatures than *P. clarkii*, which evolved in a sub-tropical climate, and so may experience less inter-seasonal fluctuation in bioturbation activity. Consequently, it is necessary to know the exact relationship between temperature and bioturbation activity for each species in order to extrapolate the data from the mesocosm experiments across the whole annual cycle and thereby better understand the overall importance of their bioturbation impacts. For *P. clarkii*, these data would also be welcome since although there are data on its bioturbation impacts from all four seasons, each season has only been sampled once and so if temperatures were particularly warm or cold for the time of year during the experiments then the extent of bioturbation and its impacts may have been over- or underestimated for that time of year in the long term. Therefore, a detailed understanding of the relationship between temperature and bioturbation activity would also help to identify the long term importance of crayfish bioturbation.

Chapters 2 and 3 demonstrate that different crayfish species produce different amounts of bioturbation, although the maximum intensity of bioturbation is difficult toi prdict without experimentation. However, if temperature is the main driver of bioturbation activity, then it should be possible to at least predict the relative annual pattern of bioturbation intensity for any given species if the relationship between temperature and bioturbation activity is known for that species. Whislt this approach would not

The exact nature of the relationship between temperature and bioturbation activity for each species is difficult to predict, however it is possible on the basis of experiments presented in the previous chapters and the information available in the literature regarding the relationship between temperature and other biological processes to hypothesise the general and relative nature of these relationships and their utility in extrapolating the mesocosm experiments, as follows:

1. The relationships between temperature and bioturbation activity for each species will likely follow an approximately logistic relationship within a range of ecologically relevant temperatures (5-25°C) as is typically seen for many rate limited biological processes.
2. The parameters of the logistic temperature relationships are expected to vary between species. In particular, they are anticipated to reflect the climatic origin of each species such that the temperate *P. leniusculus* and *A. leptodactylus* are anticipated to maintain higher relative levels of activity at low temperatures than the sub-tropical *P. clarkii.*

3. Temperature is expected to be the primary limiting factor for crayfish bioturbation activity, such that a model of the relationship between temperature and crayfish activity would be capable of accurately predicting the turbidity produced in the outdoor mesocosms based solely on temperature.
4. Predictions produced across a full annual cycle are expected to show less inter-seasonal variation for *P. leniusculus* and *A. leptodactylus* than for *P. clarkii*.

The study presented in this chapter aims to test these hypotheses by modelling the impact of temperature on bioturbation activity for the invasive crayfish species *P. clarkii*, *P. leniusculus* and *A. leptodactylus*. These models are used to predict the annual fluctuations in bioturbation activity for each species based on a 30-year average of local monthly mean temperatures and thereby enable extrapolation of the mesocosm experimental data across the entirety of an "average" year. In addition, this approach is also used to model the annual pattern of relative bioturbation intensity for the Virile crayfish, *Orconectes virilis,* in order to gain some insight into its likely bioturbation impacts. *O. virilis* was included in this study as it is an invasive crayfish species that currently has a limited global distribution, currently only being found in North America and isolated locations in the UK and Netherlands, but is expected to spread as it can successfully outcompete and displace established populations of *P. leniusculus* (James *et al.*, 2016). Consequently, investigating the likely impacts of *O. virilis* on its recipient ecosystems is of great importance and identifying its likely annual pattern of bioturbation intensity should be a useful first step in this process. Since *O. virilis* is native to northern North America and occupies similar habitats to *P. leniusculus* it is hypothesised that:

5. *O. virilis* will have a similar annual pattern of bioturbation intensity to *P. leniusculus*, with low inter-seasonal variation.

4.2 Methods

4.2.1 Experimental mesocosm setup

Six mesocosms were constructed from clear polycarbonate 10 L tanks. Dry bentonite clay (250g) was added to each tank and then all tanks were filled to capacity with tap water. The tanks were left to stand for two days with air being gently bubbled through the water to allow any sediment to settle out and for any residual chlorine in the water to evaporate. Upon wetting, the bentonite clay expanded and broke down to produce a very fine inorganic sediment that covered the bottom of the tanks to a depth of approximately 3 cm. Bentonite clay was chosen as the substrate for several reasons. Firstly, it has strong colloidal properties which mean that it is

easily put into and maintained in suspension, thereby making it useful for the study of bioturbation. Secondly, since it is inorganic it does not have any significant biological oxygen demand. This was an important design consideration since variable oxygen concentrations could impact upon crayfish activity, thereby confounding any observed effect of temperature manipulation; in addition, it meant that 100% oxygen concentrations could be maintained without the use of vigorous bubbling which would actively maintain sediment in suspension, disguising the effect of crayfish activity on turbidity. Finally, bentonite clay has previously been used as an artificial substrate to study crayfish bioturbation by Harvey et al (2014) within a similar mesocosm design.

4.2.2 Temperature manipulation

The mesocosms were set up in rooms with controlled temperature environments so that the water in the tanks could be maintained at a specific temperature for the duration of each experimental run and so that all individuals were exposed to the same temperature regime despite not being measured concurrently. Data were collected at five temperatures: 5, 10, 15, 20 and 25°C, to simulate the breadth of temperatures that would likely be experienced in a natural shallow temperate water body, such as those found in the UK. All crayfish were tested at each temperature sequentially from 5°C to 25°C to minimise any physiological stress due to large changes in temperature. In addition, each crayfish was placed in a holding tank at the appropriate temperature for six days prior to the start of experimentation to allow time for acclimatisation to the new temperature regime.

4.2.3 Measuring turbidity

Each experimental run was initiated by the addition of a single crayfish to each of the six tanks. The turbidity of the water was then measured once every minute for 16 hours with 6 turbidity probes (Partech IR40C probes connected to a Campbell Scientific CR1000 data logger), with a single probe suspended 5cm below the water surface in each tank. The probes were calibrated at 10 points with formazin FTU standards ranging from 0-600 FTU to give a sigmoidal calibration curve which was fitted with a third order polynomial function. Separate calibrations were done at each temperature since the probes exhibited a small temperature dependency. The tanks were covered with thick black polythene bags to help ensure an uninterrupted period of darkness (the period of highest activity for most crayfish species) and air was very gently bubbled near the water surface to help maintain high oxygen concentrations without creating sufficient water movement to artificially suspend sediment. To avoid conflicts with natural

circadian rhythms all experiments were run overnight. After 16 hours the crayfish were removed and the sediment left to settle out for at least 8 hours before the next experimental run was initiated.

4.2.4 Crayfish species used

In these experiments, 4 different crayfish species were used: Red Swamp (*Procambarus clarkii*), Signal (*Pacifastacus leniusculus*), Turkish (*Astacus leptodactylus*) and Virile (*Orconectes virilis*). These species were chosen for study since they are widespread invaders both in UK and globally. The first three species were also used for the experiments detailed in chapters 2 and 3 and so using the same species maximised comparability between the experiments. In addition, *O. virilis* was chosen since it is another common invasive species and by combining the results of this experiment with those from the outdoor mesocosm experiments it should be possible to estimate what level of bioturbation impacts this species would produce. A total of 12 individuals were tested for each species with a 50:50 gender ratio. The crayfish were all size matched as far as possible to a carapace length of 4-6 cm to avoid confounding species and size differences.

4.2.5 Data Analysis

During the 16 hour experimental period, most crayfish initially generated high turbidities for up to 7 hours with turbidity then dropping down to a lower plateau for the rest of the trial period. Consequently the data for each crayfish were split into two blocks of six hours, 1-7 hrs and 7-13 hrs, which were analysed separately but in like fashion in order to account for different behaviours that might be occurring over the course of the experiment. The measured turbidities for both six hour blocks were averaged for all crayfish at each temperature and pooled by species. The relationship between temperature and mean turbidity for each species was then modelled with a three parameter logistic curve, which was fitted by non-linear least squares regression. The formula for the logistic model is displayed in equation 4.1. The parameters of the model are defined as follows: Asym is the asymptotic turbidity; Xmid is the location of the inflection point and is the temperature at half Asym; and Scal is a measure of the rate of response and is the temperature increase between 50% and ≈75% Asym.

$$\text{Equation 4.1:} \qquad \text{Turbidity} = \frac{\text{Asym}}{1 + e^{\frac{\text{Xmid} - \text{Temp}}{\text{Scal}}}}$$

The logistic models were then used to predict percentage activity for each species over the course of a whole year based on 30-year averages of local mean monthly temperatures. These predictions were calibrated with known turbidity and temperature data for *P. clarkii*, *P.*

leniusculus and *A. leptodactylus* from the spring mesocosm experiments presented in chapters 2 and 3 to produce extrapolated predictions for the turbidity that would be produced by each species in the experimental mesocosms over the course of an average year. Calibration of the models was required as the physical properties of the sediment differed between the mesocosms used in these experiments and those presented in the previous chapters. In order to calibrate the models, a constant was generated for each species at both low and high crayfish densities by dividing the observed mean turbidity at each density in the spring experiments by the predicted turbidity at 13.5°C (the mean temperature during the spring experiments) generated by the model for each species. The models could then be used to predict the actual turbidity that would be produced by a given species at a given density and a given temperature. The spring data were chosen for calibration since they were available for all three species at approximately the same temperature (13-14°C).

4.3 Results

4.3.1 Species temperature curves

All four species became more active with increasing temperature, resulting in higher turbidities. There was considerable variation between individuals. However, at the species level the data exhibited logistic relationships between temperature and turbidity during both six hour blocks (Figures 4.1 and 4.2). Three parameter logistic models were successfully fitted to the data for all four species and the values calculated for the three parameters Asym, Xmid and Scal are reported in Table 4.1. The four temperature response curves were found to be significantly different from one another in both six hour blocks (ANOVA, 1-7 hrs: $F_{9,222} = 12.3$, $p < 0.001$; 7-13 hrs: $F_{9,222} = 11.5$, $p < 0.001$). The overall relationships for each species were nearly identical between the two time blocks, with the exception that asymptotic turbidity was lower for all species in the 7-13 hrs block, indicating reduced activity. The primary difference between the species response curves was the location of the inflection point (Xmid), with the temperature at half asymptotic turbidity being significantly higher for *P. clarkii* than the other species. The asymptotic turbidity was also significantly different between *A. leptodactylus* and *O. virilis* but not between the other species. *A. leptodactylus* had the highest asymptotic turbidity in both time blocks, however this is likely due to its larger body size rather than being indicative of greater bioturbation potential. *P. clarkii* asymptotic turbidity in the 7-13 hrs block was estimated with a larger standard error than the other species making it difficult to compare Asym between *P. clarkii* and the other species for that block. The third parameter Scal exhibited no difference between the four species.

4.3.2 Extrapolation of outdoor mesocosm data

The temperature response curves were employed to predict the bioturbation activity of each species over the course of a whole year using 30 year mean monthly temperatures for the local area (Met Office) as the basis for predictions. The response curves derived from the first six hour block were used for this since the crayfish were more active during this block and the standard errors of most parameters were smaller and so these models are probably more representative of the true relationships. The results of the predictions are displayed in Figure 4.3. These predictions show that *P. clarkii* bioturbation activity should vary dramatically over the course of the year, with peak in activity in July and August. *P. leniusculus* by contrast is predicted to have much less variation in turbidity with peak bioturbation activity occurring from April to October. *A. leptodactylus* activity is intermediate between these two, whilst, *O. virilis* follows a similar pattern to *P. leniusculus* only with a greater reduction in bioturbation activity during the winter months.

The shape of the predicted relationship between *P. clarkii* bioturbation activity and month is very similar to that derived from the outdoor mesocosm experiments presented in chapter 2, indicating that these temperature response models may be an effective way to predict annual crayfish bioturbation activity. This is supported by the fact that after calibration of the *P. clarkii* response curve with the mean turbidities and temperature from the spring outdoor mesocosm experiment the model accurately predicted (predictions within 95% confidence interval of mean) the mean turbidities of both crayfish density treatments in the other three experiments based solely on the average temperature recorded during each experiment (Figure 4.4). It is therefore possible for the temperature response models to be used to extrapolate the results of the outdoor mesocosm experiments across the whole annual cycle for *P. clarkii*, *P. leniusculus* and *A. leptodactylus* by using local average monthly temperature data as a basis for predictions. The predicted turbidities that would be produced by each species in the outdoor experimental mesocosms over the course of a year are displayed in Figure 4.5. The extrapolations of the data from chapter 3 for *P. leniusculus* and *A. leptodactylus* show that bioturbation activity for both species was at near maximum during the spring experiment. The peak predicted turbidity for *P. clarkii* at high density shown in Figure 4.5A is lower than that actually detected in the mesocosms (Figure 4.4) since the average temperature during the summer experiment was 5°C warmer than the 30-year average for that time of year.

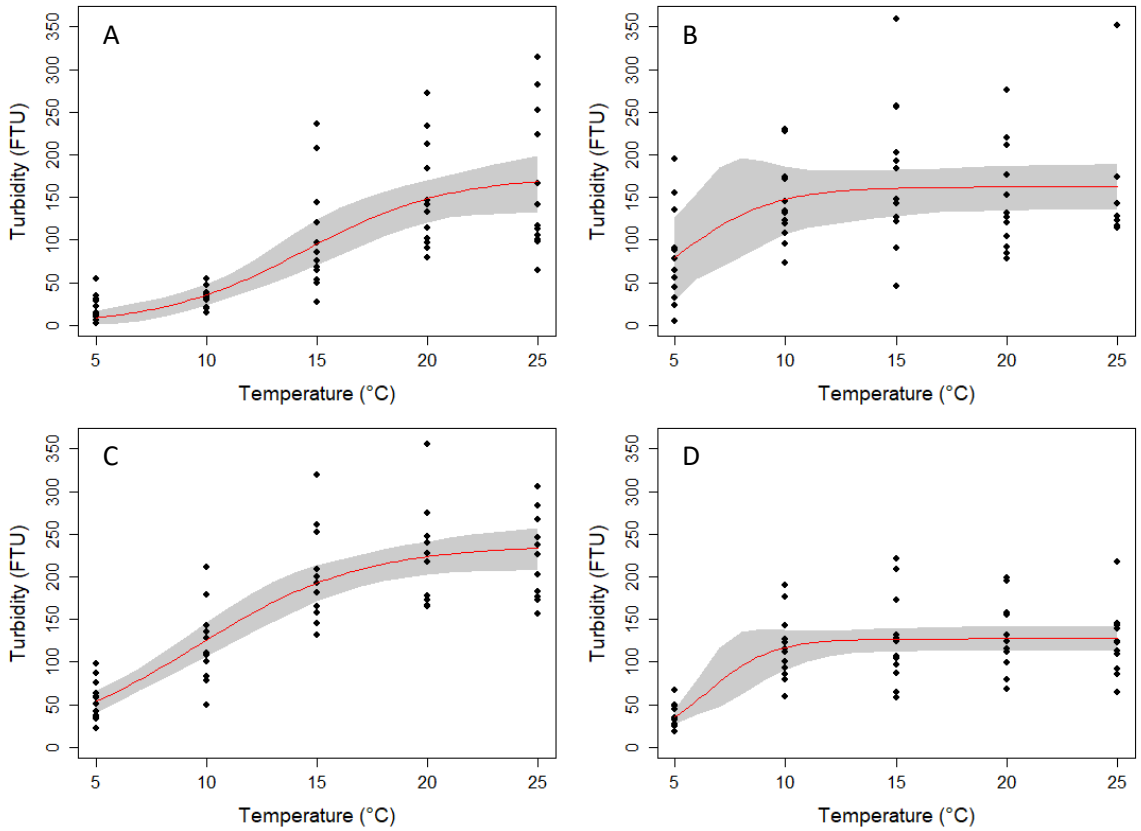

Figure 4.1: Relationship between temperature and turbidity for the 1-7 hr block.
A) *P. clarkii,* B) *P. leniusculus,* C) *A. leptodactylus* and D) *O. virilis*. Red lines are three parameter logistic models fitted using non-linear least squares regression. Grey areas represent 95% confidence intervals.

Table 4.1: Logistic model parameters for the 1-7 hr block

Species	Asym (± S.E.) (FTU)	Xmid (± S.E.) (°C)	Scal (± S.E.) (°C)
P. clarkii	175 (± 22.3)	14.4 (± 1.48)	3.25 (± 0.82)
P. leniusculus	165 (± 17.9)	5.46 (± 1.66)	3.53 (± 2.26)
A. leptodactylus	237 (± 14.8)	9.55 (± 0.88)	3.73 (± 0.64)
O. virilis	127 (± 7.17)	6.39 (± 0.63)	1.49 (± 0.59)

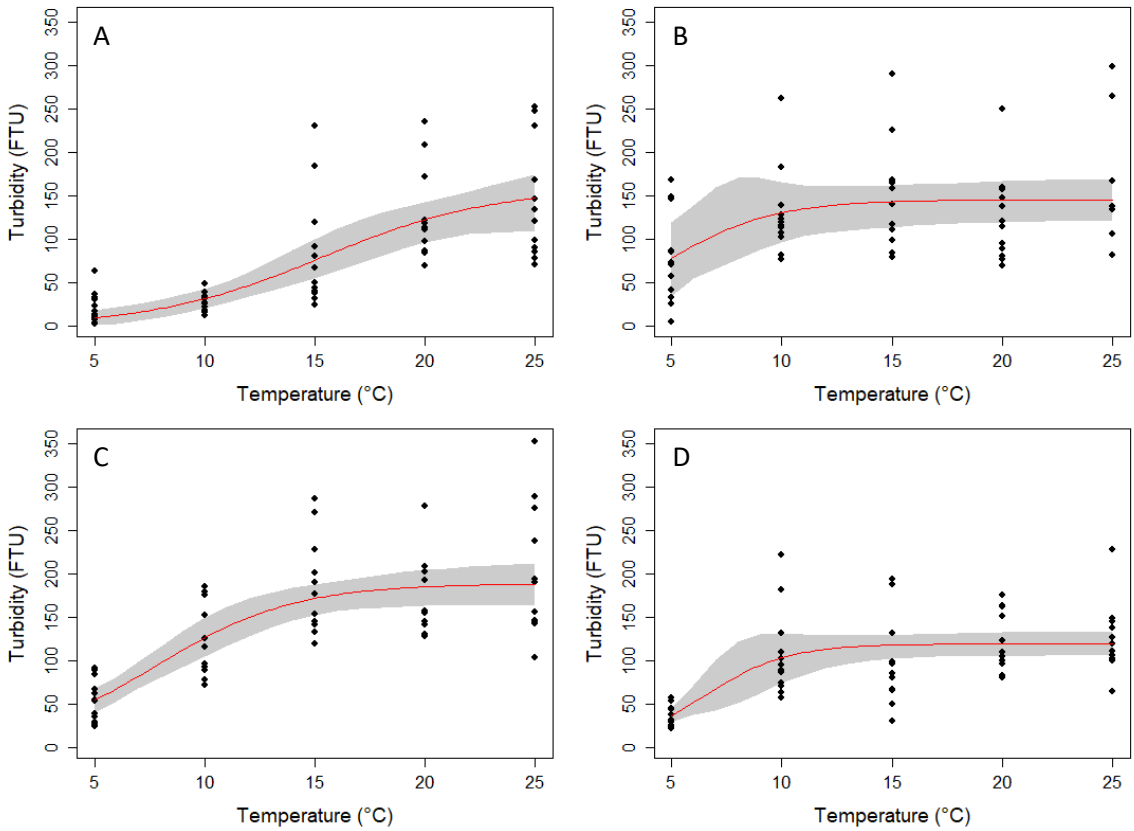

Figure 4.2: Relationship between temperature and turbidity for the 7-13 hr block.
A) *P. clarkii,* B) *P. leniusculus,* C) *A. leptodactylus* and D) *O. virilis*. Red lines are three parameter logistic models fitted using non-linear least squares regression. Grey areas represent 95% confidence intervals.

Table 4.2: Logistic model parameters for the 7-13 hr block

Species	Asym (± S.E.) (FTU)	Xmid (± S.E.) (°C)	Scal (± S.E.) (°C)
P. clarkii	159 (± 26.8)	15.3 (± 2.10)	3.84 (± 1.06)
P. leniusculus	145 (± 12.1)	4.60 (± 1.29)	2.50 (± 1.93)
A. leptodactylus	189 (± 12.7)	7.79 (± 0.79)	3.13 (± 0.70)
O. virilis	119 (± 6.88)	6.49 (± 0.72)	1.89 (± 0.80)

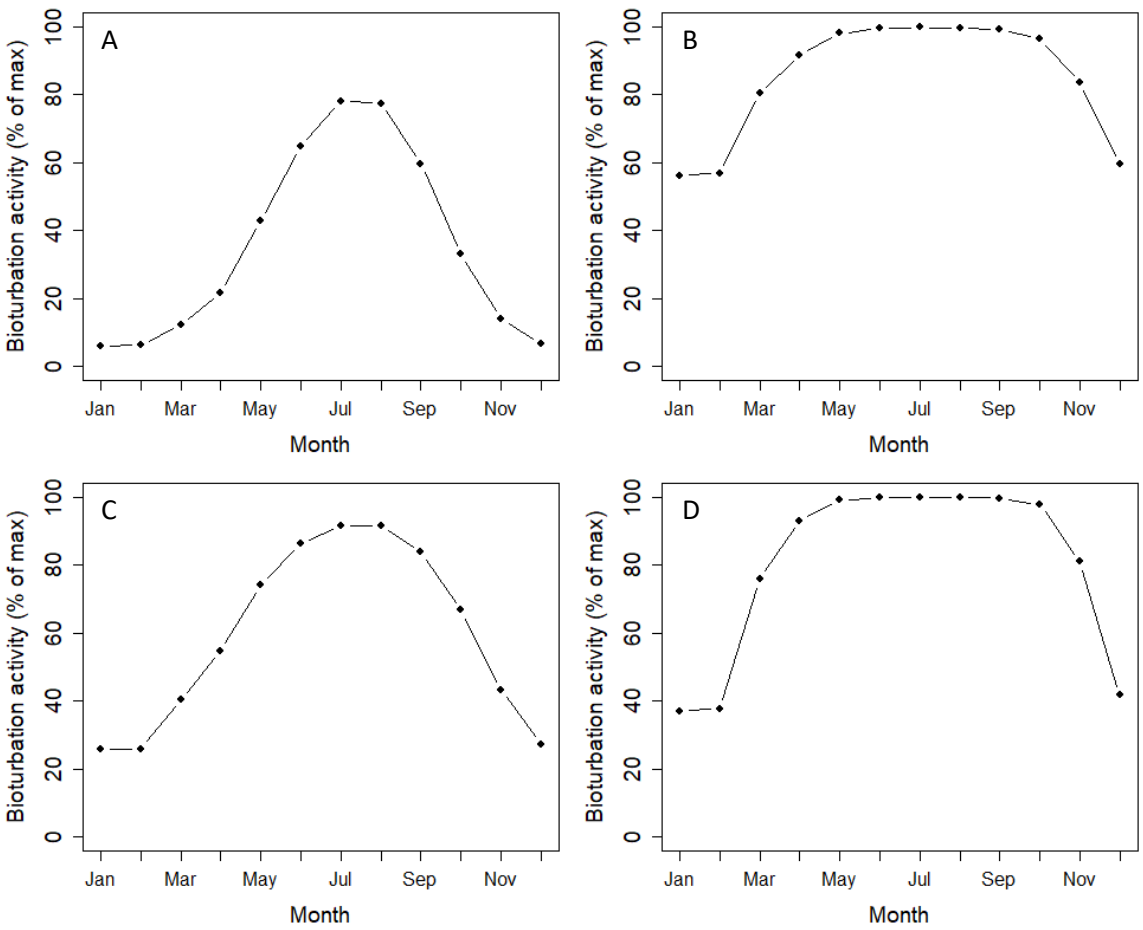

Figure 4.3: Predicted relationship between month of the year and bioturbation activity based on 30-year average of mean monthly temperature in London, UK.
A) *P. clarkii,* B) *P. leniusculus,* C) *A. leptodactylus* and D) *O. virilis*.

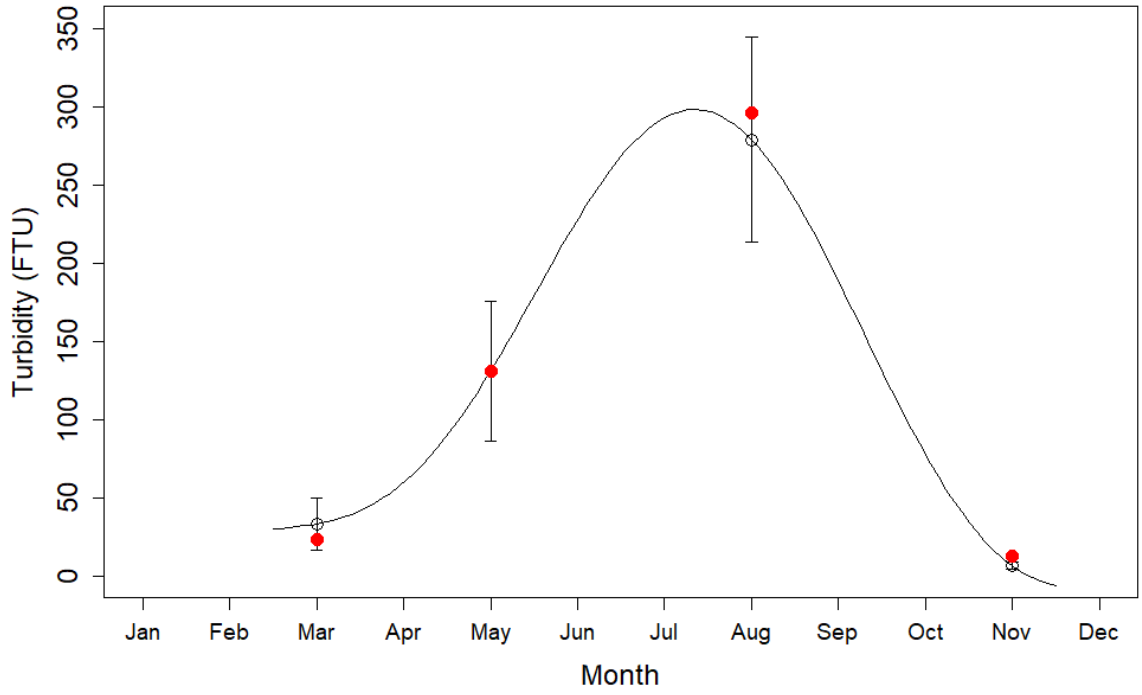

Figure 4.4: Real (open circles) and predicted (red circles) turbidities for the four high density *P. clarkii* outdoor mesocosm experiments.
Predictions based on mean temperature measured during each experiment. Error bars show 95% confidence intervals of real data values and the line is the sinusoidal model fitted to the mesocosm data in chapter 2.

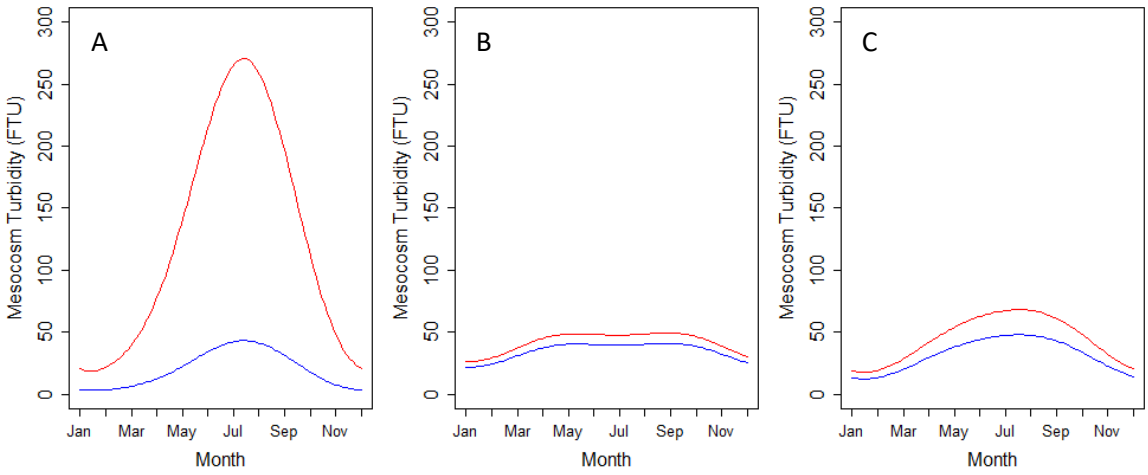

Figure 4.5: Predicted "average" relationship between turbidity and month of the year in the outdoor mesocosms for A) *P. clarkii,* B) *P. leniusculus* and C) *A. leptodactylus*.
Predictions are based on 30-year average of mean monthly temperature in London, UK. Red lines indicate high density (4 crayfish) treatment and blue lines indicate low density (2 crayfish) treatment.

4.4 Discussion

For poikilothermic organisms such as crayfish, temperature is the primary driver of metabolic rate and therefore also plays a key role in determining the intensity of activities that such an organism can undertake. The results presented in this chapter show how this principle applies to crayfish bioturbation and that the exact nature of this effect is species dependant.

In Chapters 2 and 3 it has been demonstrated that crayfish bioturbation fluctuates in a seasonal pattern. In this chapter, temperature has been investigated and identified as a primary driver of this fluctuation, however, the activity of aquatic organisms can be driven by a number of other factors which may also vary on a seasonal basis and so it is necessary to consider what influence other factors may have on crayfish bioturbation potential. Flow rate and depth are potentially important drivers of crayfish activity since crayfish are not strong swimmers and so during periods of high flow they been documented to become less active and seek refuge inside their burrows (Bubb *et al.*, 2002; Johnson *et al.*, 2014). Consequently, crayfish bioturbation intensity will likely decrease during periods of high flow, which most commonly occur the autumn and winter. This effect of seasonal variation in flows on crayfish bioturbation would not be relevant in the mesocosm experiments, as they simulate lentic environments, but in a lotic system this effect could be additive with that of temperature and serve to further decrease crayfish bioturbation activity during the autumn and winter months. Another factor that could have a seasonal influence on crayfish activity is the reproductive period. Crayfish such as *P. leniusculus* mate in the early autumn with the females then carrying the eggs on their abdomen until the next spring. As such, mate seeking behaviour during the early autumn could, to some extent, counteract reductions in activity due to low temperatures. Conversely, once females are berried, their capture rate typically declines, indicating that they become less active, which would again likely be additive with the effect of temperature on the population as a whole, serving to further reduce crayfish bioturbation activity during the winter months.

Despite the potential for other factors to have a seasonal influence of crayfish bioturbation activity, the results presented in this chapter clearly indicate that temperature is the primary driver of crayfish bioturbation intensity. It therefore seems likely that other factors would simply act as small scale modifiers on this overall pattern. This conclusion is supported by similar microcosm experiments with three different species of fish (also poikilothermic bioturbators) by Canal *et al.* (2015) which found that fish disturbed 2-3 times as much sediment at 20°C than at 10°C. It therefore appears that temperature is an excellent predictor of bioturbation intensity for poikilothermic organisms.

The relationship between temperature and crayfish bioturbation activity in these experiments conformed to a logistic curve for all four species that were tested. This type of relationship is relatively common in biological systems since there are typically multiple limiting factors for any one mechanism or process and so increasing just one of these will result in an exponential increase in the response variable until a different limiting factor becomes more important and the response will plateau. With respect to the relationship between bioturbation activity and temperature, the data for each species fitted onto a different part of the logistic curve over the temperature range that was tested. *P. clarkii* showed a pronounced sigmoidal curve; whilst *P. leniusculus* and *O. virilis* exhibited just the upper asymptotic section and *A. leptodactylus* was intermediate between the two. This indicates that *P. clarkii* activity is much more heavily limited by temperature across this range than the

other species, to the extent that at 15°C it is only at 50% of peak activity whilst the other three species are either at or near 100%. This is supported by a study from Espina et al. (1993) that found the optimal temperature of *P. clarkii* to be 23.4°C. A likely reason for this is the different evolutionary histories of these four species. The native range of *P. clarkii* lies in a sub-tropical climatic zone where the average annual temperature is around 20°C, whilst *P. leniusculus* and *O. virilis* come from more temperate climates and *A. leptodactylus* from a temperate/Mediterranean climate and so in their native ranges or climatic regions it appears that all four species would typically experience the upper asymptotic region of this relationship. Furthermore, the fact that the Scal and Asym parameters were broadly similar between the four species suggests that the rate and extent of the temperature response is essentially the same for all of them with the curve just being shifted along the temperature axis in response to each species' optimum temperature. The only species that did differ in the asymptote parameter were *A. leptodactylus* and *O. virilis*, although this was most likely in response to differences in body size and shape rather than activity since despite using crayfish with similar carapace lengths *A. leptodactylus* is naturally much wider bodied and has much larger chelae than *O. virilis* and so probably disturbed more sediment as a result.

The primary reason for conducting this study was to enable extrapolation of the data presented in chapters 2 and 3 across the whole annual cycle in order to better understand the long term impacts of bioturbation by each species. However, when looking at the temperature response curves it would appear that they are at odds with the results of the mesocosm experiments since *P. clarkii*, *P. leniusculus* and *A. leptodactylus* all attained similar maximum turbidities unlike in the mesocosms where *P. clarkii* was a much more effective bioturbator. The likely reason for this

is that the temperature experiments utilised a much shallower layer of sediment and so the only mechanisms available for sediment disturbance were walking and tail flipping but not burrowing as there was no substrate in which to burrow. Given that burrowing is an important bioturbation activity that likely varies in intensity between species, the fact that it is not accounted for in this setup means that the measurements of turbidity in these experiments should be interpreted as proxy measures of generic activity rather than bioturbation potential. Previous studies on these species have found that the rates of other activities and processes such as feeding and growth show a very similar pattern in response to temperature (Croll & Watts, 2004; Harlioğlu, 2009) and so it is likely that burrowing intensity will also conform to the same temperature response relationship and thus be predictable. Proof of this concept was provided by calibrating the *P. clarkii* response curve with data from a single mesocosm experiment and generating predictions that accurately replicated the real data. This indicates that species level responses of bioturbation activity to temperature can be successfully extrapolated from limited data by using these temperature response models.

By studying the resultant predictions based on monthly mean temperatures we can see that of the three species, *P. clarkii* is clearly expected to generate the highest turbidities for the majority of the annual cycle, despite being at somewhat less than optimal temperatures for most of the year. *P. leniusculus* and *A. leptodactylus* were estimated to be at 92% and 78% activity levels respectively during the spring experiment (mean temperature ≈14°C), meaning that turbidities generated by both species would only be expected to be slightly higher during the warmest summer months. Finally, for all three species it is evident that crayfish density is an important driver of turbidity, especially during the warmest months when the crayfish are most active. These predictions therefore support the conclusions of chapters 2 and 3 that *P. clarkii* is a more effective bioturbator than the other species and therefore would be expected to have the largest long term impact. However, it is important to note that although *P. leniusculus* and *A. leptodactylus* are predicted to produce much lower maximum turbidities than *P. clarkii,* they are still expected to cause elevated turbidities relative to crayfish free environments and to maintain these turbidities more consistently throughout the year. Therefore, they would be expected to have smaller, but perhaps more persistent, indirect impacts on ecosystem functioning, as was seen in chapter 3.

The pattern of predicted activity of *O. virilis* over the course of a year appears to be very similar to that of *P. leniusculus*, except with a slightly larger drop in activity over winter. This pattern is difficult to interpret without reference data with which to calibrate the curve, however it may be

possible to estimate the turbidity that this species would produce based on descriptions of its ecology and behaviour given in the literature. The ecology of *O. virilis* is known to be fairly similar to that of *P. leniusculus* with a preference for flowing water, high macrophyte coverage and rocky substrate (Holdich *et al.*, 2014; James *et al.*, 2016). It is known to dig pits and burrows (Bovbjerg, 1970) and therefore has the potential for significant bioturbation. However, *P. leniusculus* is similarly known to dig burrows (Guan, 1994) but is only projected to have a small impact on turbidity in the experimental mesocosms and so if the extent of burrowing by *O. virilis* is limited, as reported by Bovbjerg (1970), then it seems likely that *O. virilis* would generate a similar annual turbidity curve to *P. leniusculus*. A minor caveat to this would be that *O. virilis* is reported to be more aggressive than *P. leniusculus* (James *et al.*, 2016) and so greater amounts of inter- and intra-specific agonistic behaviour may serve to increase its bioturbation potential.

Chapter 5: Effect of manipulation of crayfish density on bioturbation impacts in a lowland chalk stream

5.1 Introduction

In the previous chapters I have shown that invasive crayfish species have the potential to impact upon numerous ecosystem properties and processes in an experimentally controlled environment using mesocosms; however, the true applicability of these results to a real world scenario remains untested. Mesocosms are useful experimental systems since they allow a high degree of control and replication, but this also means that they are inherently simplified ecosystems both in terms of complexity and scale and so direct extrapolation to real world scenarios is difficult since the relative importance of any identified relationships may be over- or underestimated (Carpenter, 1996; Englund & Cooper, 2003). For example, the mesocosms used in the experiments detailed in chapters 2 and 3 represent only one type of habitat with a specific sediment composition and water to sediment ratio and so the extent of crayfish bioturbation in heterogeneous environments is difficult to estimate. In addition, a natural system is likely to have other bioturbation sources such as benthic fish that could conceal any effect of crayfish bioturbation (Schaus & Vanni, 2000). An approach that has proved useful when interpreting other mesocosm experiments is to compare the results with data collected from a variety of appropriate natural systems (Ledger *et al.*, 2009; Brown *et al.*, 2011). By looking for the same relationships that have been previously characterised in the mesocosms it is then possible to understand their relative importance in a natural system. In this chapter, I present just such a study conducted on a natural river system in order to aid interpretation of the results presented in the previous chapters.

However, the collection and interpretation of the appropriate field data is challenging since the conditions within a natural water body tend to be difficult, time-consuming or impractical to experimentally alter, whilst replication across multiple different water bodies tends to be confounded by variations in their biotic and abiotic characteristics (Schindler, 1998). For instance, in Chapters 2 and 3 the observed effects of crayfish bioturbation were heavily dependent on crayfish density and so the best way to identify any effect of crayfish bioturbation in a natural system would be to sample and compare multiple sites with different crayfish densities. However, the fact that it is extremely difficult to get any quantitative measure of crayfish density in a natural system (Momot *et al.*, 1978), and that other factors that might also

influence the intensity of bioturbation and its impacts, such as fish population size, community species composition, sediment type or water depth, also vary between study sites means that isolation of a crayfish bioturbation effect from such data would be challenging. Consequently, this study was designed to minimise these issues by experimentally manipulating crayfish density within a single site, thereby generating a known gradient in crayfish density while retaining uniformity of physical characteristics.

The study site chosen for this experiment was a lowland chalk stream with a well established population of signal crayfish, *Pacifastacus leniusculus*. This site is not a direct analogue of the mesocosms used for the previous work since it is a lotic rather than lentic system but was chosen for a number of reasons. *P. leniusculus* is the most widespread invasive crayfish species in the UK, and other temperate regions, and so understanding its bioturbation impacts is of primary importance. In addition, chalk streams are a globally rare habitat in which mobilisation of fine sediment is already of great concern (Wood, 1997; Walling & Amos, 1999) and with the majority being found in southern England (Berrie, 1992), where *P. leniusculus* is very common (Holdich & Reeve, 1991; Holdich *et al.*, 2014), determining the impacts of *P. leniusculus* bioturbation in chalk streams is of additional importance. Previous studies on crayfish bioturbation have primarily focused on *P. leniusculus* in a stream environment and have found evidence that this species can cause significant sediment disturbance, including an increase in turbidity, in lotic systems (Johnson *et al.*, 2010; Harvey *et al.*, 2011, 2014). As a result, using this site allows the results of this study to be more easily placed in the context of the wider literature. Finally, it is more practicable to manipulate crayfish density in a short stretch of stream than within a pond or lake and so it is a viable first step to identify any crayfish bioturbation effects in a natural system.

The most significant effects of crayfish density observed in the mesocosm experiments were on turbidity, dissolved oxygen concentration and methane oxidation potential in the water column; therefore, this study aims to address the following hypotheses:

1. Manipulation of crayfish density in the stream should affect bioturbation intensity, such that turbidity would be lower in a section with reduced crayfish density and higher in a section with elevated crayfish density.
2. Changes in crayfish bioturbation intensity should alter the amount of suspended organic matter and therefore the biological oxygen demand of the water column, such that dissolved oxygen concentrations in the water column would be expected to decrease with increasing crayfish density / turbidity.

3. Changes in crayfish bioturbation intensity would also be expected to impact the suspension of methane oxidising bacteria from the sediment into the water column, such that the methane oxidation potential of the water column would increase with increasing crayfish density / turbidity.

5.2 Methods

5.2.1 Study site and experimental design

This experimental study was conducted in a 200 m stretch of Chalgrove Brook, a tributary of the River Thame in Oxfordshire. The study ran for 8 weeks from August to October 2015. Chalgrove Brook is classified as a lowland chalk stream with permanent flows. The experimental reach had a channel width of 2-3 m and a water depth of 50-75 cm. There were few submerged macrophytes and the bed material was approximately 50% gravel and 50% silt. It was chosen for study since it was known to host a well established population of *P. leniusculus*, (pers comm. B Campbell, landowner) and the local landowner was willing to provide access and allow experimental manipulation of the stream.

Three sequential 50 m enclosed sections of the stream were created by constructing four 1 m high fences across the channel out of 5 mm wire mesh supported by steel poles (Figures 5.1 and 5.2). The fences were secured tightly against the bed and banks to impede crayfish movement up or down stream. The fences were cleared of leaves and other debris at least twice a week to prevent changes in water flow or depth. 50 crayfish traps were deployed evenly across the central section and emptied 2-3 times per week in order to reduce crayfish density in this section. This was confirmed by a decline in catch per unit effort (CPUE) over time. All crayfish caught were tallied to measure CPUE and then released into the downstream section to increase the crayfish density in that section. The upstream section was left unmodified. This process was designed to generate three near identical stretches of stream differing only in crayfish density; the three densities being normal (upstream), low (central) and high (downstream). The relative densities were confirmed by placing 16 traps in each section simultaneously for 48 hours to determine CPUE in each section immediately after data collection was concluded.

Figure 5.1: Picture of exclusion fence between downstream and central section.
Crayfish attempting to climb fence indicates fence is relatively effective at impeding movement within the stream channel.

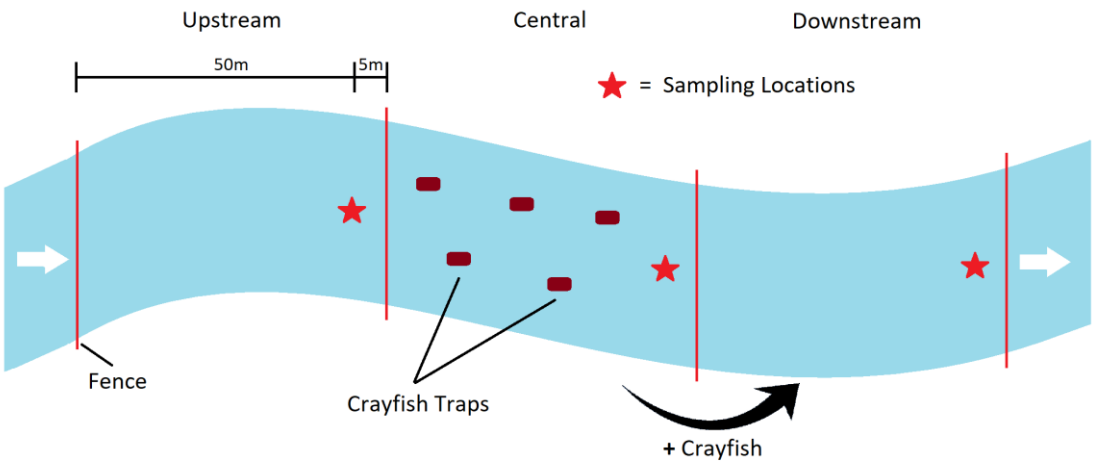

Figure 5.2: Schematic showing experimental design.
50 crayfish traps were deployed in the central section.

5.2.2 Measurements

Turbidity was measured every 5 minutes over a 24 hour period using 3 Partech IR40C turbidity probes connected to a Campbell Scientific CR1000 data logger. The probes were suspended in the centre of the channel 20 cm above the sediment surface. Measurements were taken during week eight of the experiment since CPUE had ceased to decline and were collected on three separate days (A, B and C) in order to allow for variation in environmental factors, such as temperature or flow speed, occurring over time scales longer than 24 hours. On each occasion that turbidity was measured, a spot measurement of dissolved oxygen concentration was also taken at 10:00 AM using 2 Unisense OX-10 oxygen microsensors attached to a Unisense UnderWater data logger. At the same time, water samples from each section were collected from ≈20 cm above the sediment surface with a gas-tight 50 ml syringe. The water sample from each section was discharged into six gas-tight 12.5 ml glass vials until overflowing and capped. The samples were then returned to the laboratory and three from each section were prepared for dissolved methane analysis as described in section 2.2.4 and the other three were prepared for methane oxidation analysis as described in section 2.2.5. All samples and measurements were taken 5 m upstream of the downstream fence of each section (Figure 2.2). Two weeks after completion of the experiment and removal of the fences turbidity was again measured at all three locations to test for variation due to factors other than experimental treatment.

5.2.3 Data analysis

All data collected were tested for differences between sections using ANOVA with type II Sums of Squares. Since data were collected on three separate occasions all statistical analyses used linear mixed effects models with sampling date as a random effect to account for variation between sampling occasions. The median rather than mean of each 24 hour period was used for analysis of turbidity since the data contained several large spikes which were likely due to debris rather than sediment. All statistical analyses were done using R statistical software (R Development Core Team 2017).

5.3 Results

5.3.1 CPUE

Over the course of eight weeks of trapping, a total of 2574 crayfish were removed from the central section and added to the downstream section. CPUE declined over this time in accordance with a negative power relationship (Figure 5.3) although CPUE did not decline all the

way to zero indicating that crayfish were still present in the central section albeit at a reduced density. A mark-release-recapture experiment conducted with 500 of the captured crayfish found that 10% of relocated crayfish were recaptured in the central section, indicating that the fences were not completely successful at stopping crayfish movement. However, measurements of CPUE in each section one week after all data collection had been completed show that crayfish densities in the central and downstream sections had been successfully depleted/elevated relative to the control upstream section (Table 5.1).

5.3.2 Turbidity and dissolved oxygen

Turbidity was significantly different between the sections ($F_{2,4}$ = 13.1, p = 0.02) with the high density section consistently having the highest turbidity (Figure 5.4). However, the estimated size of the effect of crayfish density on turbidity was very small with the low and normal density sections being only 2.5 FTU and 2 FTU lower than the high density section respectively. Such small differences are close to the detection limit for the turbidity probes and so this result should be interpreted with caution. After the fences were removed and thus crayfish densities equalised there was no significant difference in turbidity between the three locations ($F_{2,6}$ = 0.02, p = 0.98; Figure 5.4).

Dissolved oxygen concentrations were not significantly different between the sections ($F_{2,4}$ = 2.4, p = 0.2) with mean oxygen saturation across all sections and on all sampling occasions at 73% (Figure 5.5).

5.3.3 Dissolved methane and methane oxidation

Dissolved methane concentrations were oversaturated relative to atmospheric equilibration (3.2 nmol L^{-1} at 10°C) with a mean of approximately 44 nmol L^{-1}. Crayfish density was found to have no significant effect on methane concentrations.

Methane oxidation potential of the water column (MOP_{wat}) was not significantly different between the sections ($F_{2,4}$ = 0.88, p = 0.5). Mean MOP_{wat} across all sections and sampling occasions was 0.14 µmol L^{-1} h^{-1} (Figure 5.6).

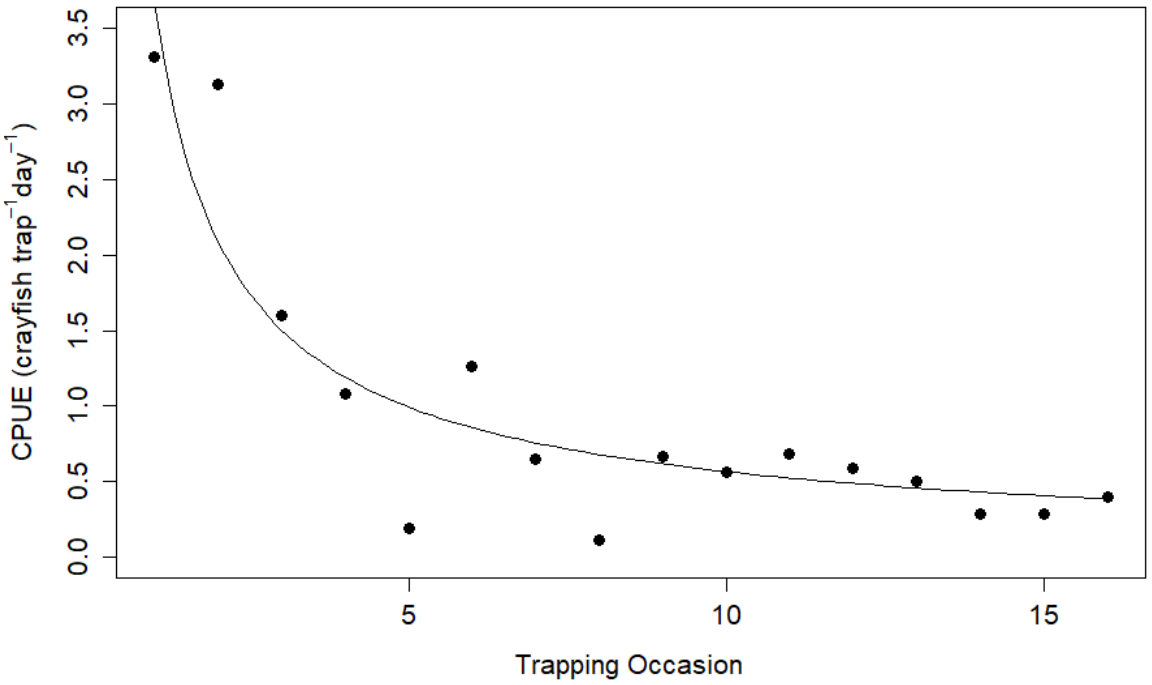

Figure 5.3: CPUE in central section over the course of eight weeks.
Traps were emptied twice per week. CPUE declined over time, conforming to an inverse power relationship (solid line) with the formula: $y = 3.7x^{-0.8}$

Table 5.1: CPUE in each section at end of experiment

Section (Density)	CPUE (Crayfish trap^{-1} day^{-1})
Upstream (Normal)	0.45
Central (Low)	0.32
Downstream (High)	1.12

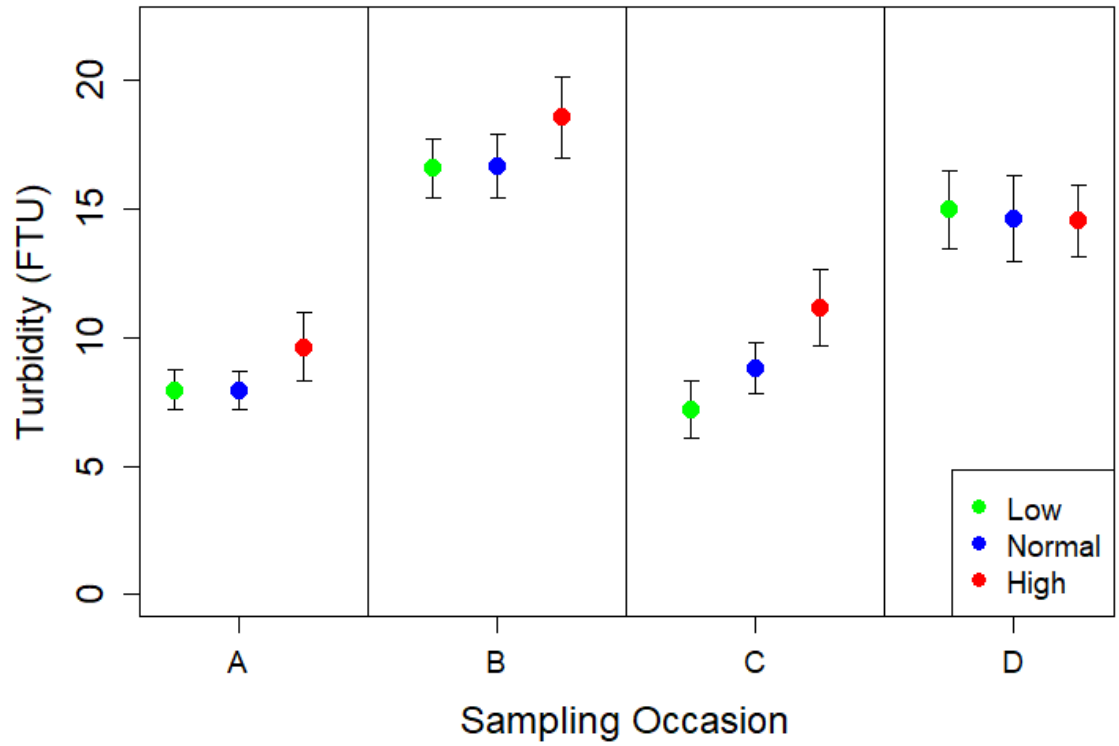

Figure 5.4: Mean turbidity ± SE for each sampling occasion.
Sampling occasions A-C were with the fences in place and D was after fences were removed. Red is high density (downstream) section, blue is normal density (upstream) section and green is low density (central) section.

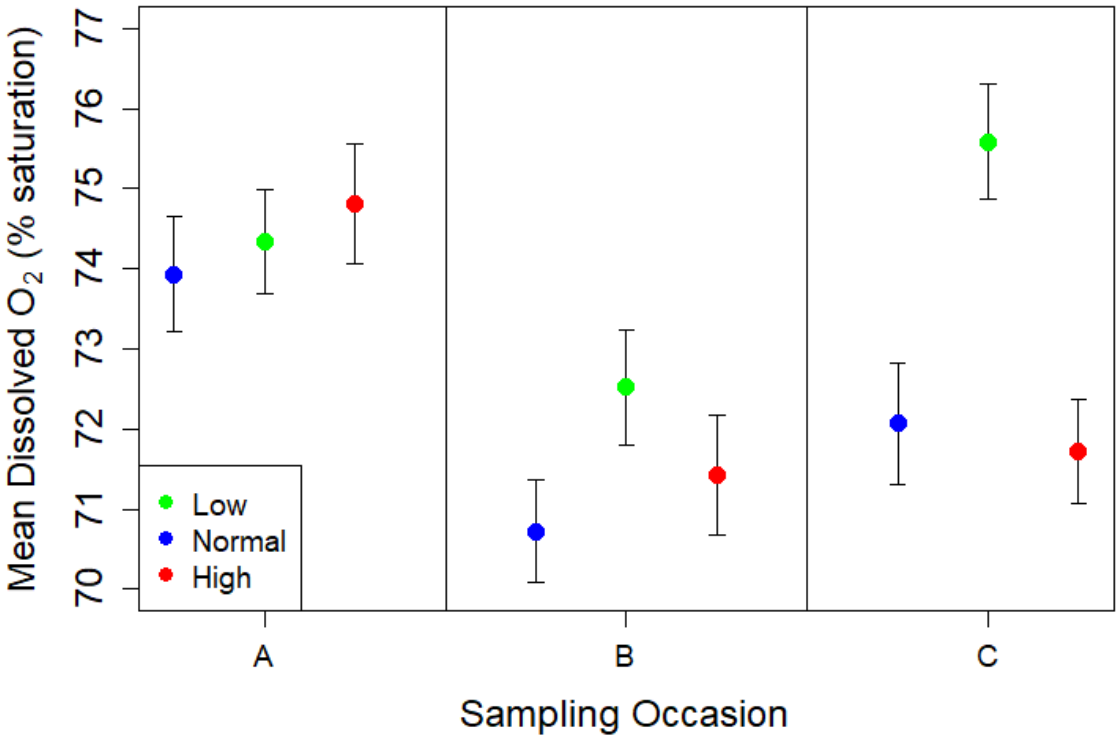

Figure 5.5: Mean dissolved oxygen saturation ± SE for each sampling occasion.
Red is high density (downstream) section, blue is normal density (upstream) section and green is low density (central) section. Overall mean is 73%.

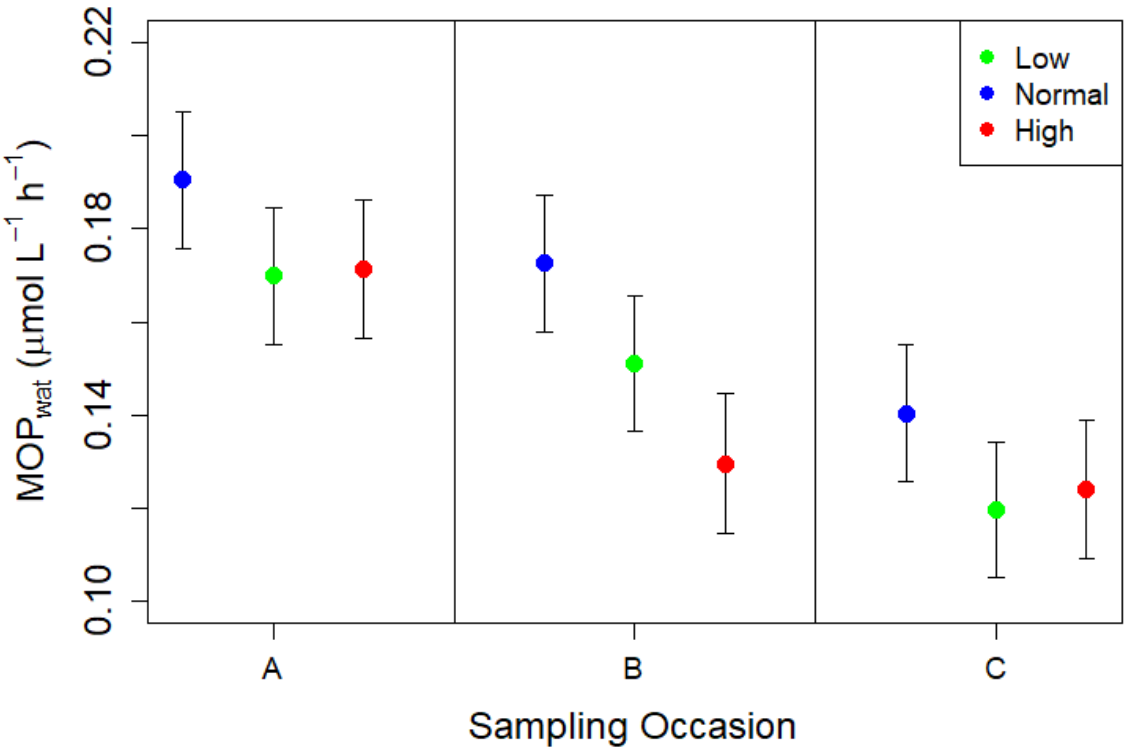

Figure 5.6: Mean methane oxidation potential of the water column ± SE for each sampling occasion. Red is high density (downstream) section, blue is normal density (upstream) section and green is low density (central) section.

5.4 Discussion

Crayfish density was found to have a significant and positive relationship with turbidity in the Chalgrove Brook; however, it was not found to correlate with changes in dissolved oxygen concentrations or methane dynamics. CPUE in the central section did not decline to zero, which means that crayfish were still present in this section, albeit at a lower density, and so a true crayfish-free control could not be generated. The failure to completely eliminate crayfish from the central section results from the fact that trapping efficiency declines along with population density so that some individuals will always escape capture (Gherardi *et al.*, 2011). In addition, the mark-recapture results showed that approximately 10% of translocated crayfish managed to re-colonise the central section, indicating that the fences were not completely effective at stopping crayfish movement. Despite this, the measurements of CPUE in each section clearly show that three different crayfish densities (low, normal and high) were generated in the three sections, such that this setup could be used to test for the same effects that were observed in the mesocosm experiments.

The primary effect of crayfish density that was observed in the mesocosms was on turbidity and this was replicated to a small extent in the real world environment of this experiment. The differences in turbidity detected between the treatments in this stream experiment were however very small, falling very close to the detection limit of the turbidity sensors. It is therefore difficult to say for certain if the detected differences are in fact real or just an artefact generated by instrument error. Nevertheless, the results of this experiment do suggest that crayfish may be responsible for some sediment resuspension in this stream. This conclusion is supported by the fact that once the fences were removed and thus crayfish were free to move and equalise their density across the three sections the turbidity became almost identical at all three sampling locations. Additional support comes from other studies that have linked *P. leniusculus* activity with sediment disturbance in rivers. Harvey *et al.* (2014) found that in lowland rivers that are home to *P. leniusculus*, turbidity exhibits diel fluctuations that coincide with patterns of *P. leniusculus* activity, indicating that this species is capable of producing significant sediment resuspension in a stream environment, especially overnight. However, no evidence of diel patterns in turbidity was found during this experiment, which again indicates that any effect of crayfish activity on turbidity was likely to be very small. Nevertheless, Rice *et al.* (2016) estimate that during baseflow conditions, *P. leniusculus* may contribute at least 32% to suspended sediment load in stream conditions, which corresponds relatively well with the results from this study which found that the high crayfish density section had an average

increase in turbidity relative to the low density section of 24%. Whilst this value is lower than that reported by Rice *et al.*, it is important to note that there were still crayfish present in the low density section that would still have been causing an amount of sediment disturbance and so it is likely that in a truly crayfish-free environment the turbidity would be lower still.

Although the high density section had a 24% higher turbidity than the low density section the absolute magnitude of the difference was small at only 2.5 FTU. Whilst comparison with a completely crayfish-free environment would probably generate a larger turbidity differential, the low turbidities produced in this experiment would still keep the absolute difference small. Given that in the experimental mesocosms this species generated a peak turbidity differential of 20 FTU and this was associated with only weak effects on dissolved oxygen and methane oxidation it is unlikely that such a small increase in turbidity will have significant biological effects. One possible reason for the low turbidities is that these data were collected at the end of September and beginning of October when temperatures were beginning to cool and hence the crayfish may have become less active. Indeed, the mean water temperature during data collection was 9.5°C which is below the optimal bioturbation temperature of approximately 13°C for this species as demonstrated in chapter 4. In addition, the effect of low temperatures on activity may have been compounded by other factors. In particular, females carrying eggs (berried) are far more abundant at this time of year and catch per unit effort for berried females is in my experience generally much lower than for un-berried females. This indicates that females become less active when berried, which would likely further reduce bioturbation activity at this time of year. Furthermore, other factors known to decrease crayfish activity that would become more prevalent at this time of year, such as high flow rate and depth (Bubb *et al.*, 2002; Johnson *et al.*, 2014), may have also reduced crayfish bioturbation intensity. Consequently it may well be the case that during the summer (when it is warmer, the crayfish are not breeding and there are lower flows) the turbidity differences between the sections would be larger. It is also interesting to note that on one sampling occasion (C) the turbidity in the low density section was significantly lower than the normal density section 50 m upstream. This indicates that sediment may remain in suspension for only short distances downstream, with bioturbation by downstream crayfish replacing sediment that settles out, so that impacts of crayfish bioturbation may be very localised according to patterns of crayfish abundance.

In the experimental mesocosms the dissolved oxygen concentration was strongly affected by crayfish bioturbation but this relationship was not replicated in this study. Whilst this does not agree with the mesocosm work, this result is to be expected since turbulence and mixing in a

flowing system act to continuously replenish dissolved oxygen and so would buffer against any drawdown due to variable oxygen demand between the sections generated by small differences in suspended sediment load. Indeed, oxygen saturation remained constant at 73% ± 3% across all sections and sampling occasions. By contrast, in the experimental mesocosms there was no flow other than wind mixing of the surface and so dissolved oxygen was primarily derived from that generated in situ by photosynthesis during the day and so differences in oxygen demand could be magnified by overnight drawdown. The fact that oxygen saturation in the stream was less than 100% despite the turbulent mixing effect indicates that something (e.g. organic pollution) is already drawing down oxygen concentrations. It is therefore possible that if flow rates were lower, or the turbidity higher, oxygen concentrations could be impacted by crayfish bioturbation since the oxygen buffering capacity may already be saturated. Possible evidence for this is supplied by the fact that the largest difference in oxygen saturation between the low and high density sections (+4.9%) occurred on sampling occasion C, when the difference in turbidity was also at its greatest. Overall however, it would appear that crayfish bioturbation impacts on dissolved oxygen may be of more importance in habitats with low or no flow such as ponds, lakes and backwaters, although further experimentation would be necessary to confirm this.

Dissolved methane concentrations were significantly oversaturated relative to atmospheric equilibrium but fell within values reported for chalk streams by a previous study (Shelley *et al.*, 2014). The rates of methane oxidation in the water column (MOP_{wat}) by methane oxidising bacteria were similar to those observed in the experimental mesocosms stocked with *P. leniusculus* and to those reported for other chalk streams (Trimmer *et al.*, 2009; Shelley *et al.*, 2014), although there was no significant relationship with crayfish density in this experiment. The lack of any effect on MOP_{wat} is likely due to the fact that the difference in turbidity was very small between the sections since in the mesocosms a difference of at least 20 FTU was required to detect an effect on MOP_{wat}. The reason for this is that errors in estimation of MOP were relatively large and since re-suspended sediment is the primary vector that carries additional methane oxidising bacteria up into the water column under bioturbation it requires quite large differences in turbidity to generate a statistically significant difference in MOP_{wat}. It is also possible that the flow was sufficiently high to prevent settling out of suspended bacteria in the low density section, thereby reducing differences between the sections even further. As with dissolved oxygen, it would be necessary to conduct further study on this in lentic environments to determine the wider importance of this effect.

This study has provided further evidence that *P. leniusculus* does increase turbidity in a stream environment through sediment resuspension but has not provided supporting evidence for other potential impacts of crayfish bioturbation that were identified in the mesocosm experiments. This failure to replicate the results of the mesocosm experiments may be due to a number of causes. One possibility is that the experimental design was simply not strong enough and failed to account for certain ecosystem properties or processes that had countervailing effects on crayfish bioturbation. For instance, *P. leniusculus* has been documented to burrow preferentially in certain types of soils and to burrow less frequently if other means of shelter are already available (Guan, 1994). As such, local abiotic conditions may have been a more important driver of crayfish bioturbation activity than population density. Alternatively, it is possible that the specifics of this experiment may simply be too different from the mesocosm experiments to yield comparable results. For instance, as has been previously discussed, drivers of crayfish bioturbation activity and sediment suspension in general may be very different in lotic versus lentic systems. Finally, the fact that this experiment was only run on one occasion, in one river and at a time of year with reduced activity means that interpretation of this result is difficult, as it is unlikely to be entirely representative of the situation in other rivers or at other times of year. It is therefore recommended that further study be done, particularly in lentic habitats and at other times of year to fully identify the real world implications of the mesocosm results.

Chapter 6: Conclusions

6.1 Overview

The data presented in the previous chapters demonstrates that bioturbation by invasive crayfish species can have ecologically important impacts on their recipient ecosystems. In artificial mesocosms the intensity of bioturbation, and thus the extent of such impacts, increased with crayfish density and varied between crayfish species. Red Swamp crayfish, *P. clarkii*, were found to be extremely active bioturbators during the spring and summer, whilst Signal crayfish, *P. Leniusculus*, and Turkish crayfish, *A. leptodactylus*, were found to be much less effective, producing only 25% as much turbidity as *P. clarkii* during the spring. Crayfish bioturbation was found to negatively impact dissolved oxygen concentrations, to increase algal biomass and to alter zooplankton community structure. Methane dynamics were also affected by crayfish bioturbation with methane oxidation in the water column and the potential utilisation of methane derived carbon by the pelagic food web being particularly affected. Intensity of crayfish bioturbation varied seasonally such that impacts detected for each species during the summer months were either much smaller or undetectable during the winter months. Experiments with controlled temperature manipulations demonstrated that this seasonal variation was most likely in response to seasonal changes in temperature, which enabled extrapolation of the mesocosm results across the whole annual cycle. *P. Leniusculus* was found to be producing bioturbation in a natural river system, however there was a lack of detectable knock-on impacts. Nevertheless, it may be that at other times of year, in other types of freshwater systems or for other species, that this bioturbation may produce more ecologically significant impacts.

6.2 Invasive crayfish species can cause significant bioturbation resulting in widespread ecological consequences

The basic theme that underlies all four data chapters is that the three invasive crayfish species: *P. clarkii*, *P. Leniusculus* and *A. leptodactylus*, cause extensive bioturbation that can be detected as a significant increase in turbidity (i.e. a higher concentration of suspended sediment). In chapters 2 and 3 I utilised an experimental mesocosm approach to conclusively demonstrate this and to characterise the knock-on or indirect impacts of bioturbation by these species for the wider ecosystem. It is clear from this work that all three crayfish species can produce significant bioturbation and that it increases in intensity with population density.

Crayfish bioturbation was found to have several impacts in the experimental mesocosms. Perhaps the most ecologically important impact was that increasing bioturbation intensity both within and between species resulted in reduced oxygen concentrations. This impact was sufficiently strong that the highest intensity bioturbation observed was capable of generating harmful overnight hypoxic conditions in the mesocosms. In addition, this work demonstrated that crayfish bioturbation can promote algal growth through accelerated decomposition to the extent of triggering algal blooms and cause changes in zooplankton community structure. These impacts are inevitably interrelated and subject to feedback loops, but it is clear from this work that invasive crayfish bioturbation has the potential to cause severe and potentially undesirable impacts on recipient ecosystems.

The work in chapter 2 also demonstrates the potential for crayfish bioturbation to affect ecosystem functioning, particularly methane dynamics. Of greatest significance was a large increase in the methane oxidation potential of the water column in response to high intensity bioturbation, indicating an increased population of pelagic methane oxidising bacteria. The association of this with increased depletion of ^{13}C in common filter feeding zooplankton suggests that high intensity bioturbation may increase the importance of methane derived carbon to the pelagic food web.

6.3 The pattern and intensity of bioturbation and thus severity of impact varies between species

Crayfish bioturbation intensity fluctuates seasonally with corresponding fluctuations in the magnitude of its impacts. In chapter 2 this was very clearly demonstrated for *P. clarkii* where significant bioturbation and its impacts were only detected in the spring and summer months. However, given the differences in ecology, physiology and behaviour between species, the magnitude and timing of the seasonal fluctuations is species specific. In chapter 3 I repeated the experiments of the previous chapter with *P. Leniusculus* and *A. leptodactylus* in order to verify this. This work showed that at two separate times of year *P. Leniusculus* and *A. leptodactylus* consistently generated significantly different levels of bioturbation to *P. clarkii*, indicating that the seasonal pattern of bioturbation varies between these species.

The most common driver of seasonal patterns of activity in poikilothermic organisms is temperature. In chapter 4 I utilised a different experimental mesocosm approach to characterise the impact of temperature on bioturbation intensity for all three experimental species. This work showed that *P. clarkii* activity is much more strongly affected by low temperatures than *P.*

Leniusculus or *A. leptodactylus*, indicating that in a temperate climate, seasonal fluctuations in bioturbation intensity will be proportionally larger for *P. clarkii*. Species specific temperature response models enabled estimation of a full annual pattern of bioturbation intensity for each species. Comparison with results from chapters 2 and 3 verified that temperature is the primary driver of crayfish bioturbation intensity and predicted that peak bioturbation intensity during the summer would be far lower for both *P. Leniusculus* and *A. leptodactylus* than for *P. clarkii*. Consequently, the ecological impacts of *P. clarkii* bioturbation are likely to be the most severe of the three experimental species. However, bioturbation by *P. Leniusculus* and *A. leptodactylus* is predicted to be more consistent throughout the year and so whilst their bioturbation impacts may be smaller, they may also be more persistent.

6.4 *P. Leniusculus* generates significant bioturbation in a natural chalk stream environment but additional impacts were not detectable

The extremely simplified nature of experimental mesocosms renders the extrapolation of conclusions drawn from mesocosm experiments a difficult task. In chapter 5 I experimentally manipulated *P. Leniusculus* density in a natural chalk stream in order to test the major conclusions drawn from the mesocosm experiments. This work supported a primary conclusion that *P. Leniusculus* can produce detectable bioturbation in a stream environment, although its intensity was much lower than was observed in the mesocosms. However, additional indirect impacts on dissolved oxygen and methane oxidation were not detected. The reason for this is likely to be that the bioturbation intensity was low and that the higher flow rate in a stream helps to maintain oxygen concentrations and reduce settling out of suspended particles and bacteria. Therefore these results do not provide much direct support for the conclusions drawn from the mesocosm experiments, although this may be due to the limitations of the experiment rather than indicating that those conclusions are not applicable to a stream environment. As such, in an environment with lower flow, in a different river or at higher temperatures this might not be the case. In addition, the species used for this experiment, *P. Leniusculus*, was found to have the least severe bioturbation impacts of the three species tested in the experimental mesocosms and so the other species may be expected to have a greater impact. Given all this, the fact that it was simply possible to detect *P. Leniusculus* bioturbation as a change in turbidity in an environment so different to that of the mesocosms demonstrates that crayfish bioturbation is a widespread and powerful ecosystem engineering process and so the applicability of the mesocosm experiments to other situations cannot be dismissed and is in need of further investigation.

6.5 Recommendations for further work

In this thesis I have presented novel work that expands our knowledge of the indirect impacts of invasive crayfish bioturbation and on the basis of this work I have identified a number of knowledge gaps that would be suitable targets for further study.

In chapters 2 and 3 I found that crayfish bioturbation increased rates of methane oxidation in the water column and that this may result in the increased importance of methane derived carbon to the pelagic food web. If this is the case, it would represent an important advance in our understanding of how freshwater systems function. Accordingly, I would recommend further work to confirm this result. In particular, I would recommend using lipid or molecular analysis of zooplankton and possibly other organisms at higher trophic levels to directly measure the extent to which uptake of methane derived carbon by the pelagic food web is affected by bioturbation.

In chapters 2, 3 and 4 I demonstrated that different crayfish species produce different amounts and patterns of bioturbation and that the severity of the knock-on impacts therefore varies accordingly. However, I only generated detailed information on this for three out of over 20 species of crayfish that are known to be invasive on a global scale. As such I believe there is scope for further similar studies utilising other invasive crayfish species in order to more fully understand the impact of crayfish bioturbation on a global scale.

Finally, in chapter 5 I attempted to corroborate the results of the mesocosm experiments with a larger scale *in-situ* experiment within a real-world stream, although the results of this experiment were inconclusive. As such, I would recommend further ecosystem scale experiments to more fully understand the true importance of the potential impacts of crayfish bioturbation identified by the mesocosm experiments within real-world ecosystems. In particular, I would recommend repeating the same experiment (or similar) during the summer and in a variety of different streams. In addition, I would recommend trying to conduct similar experiments in, or surveys of, lentic systems.

Chapter 7: Bibliography

Alderman D.J., Holdich D. & Reeve I. (1990) Signal crayfish as vectors in crayfish plague in Britain. *Aquaculture* **86**, 3–6.

Anastácio, P. M., Correia, A. M., Menino, J. P. & Martins da Silva, L. (2005) Are rice seedlings affected by changes in water quality caused by crayfish? *Ann. Limnol. - Int. J. Lim.* **41**, 1–6.

Anderson C.B. & Rosemond A.D. (2007) Ecosystem engineering by invasive exotic beavers reduces in-stream diversity and enhances ecosystem function in Cape Horn, Chile. *Oecologia* **154**, 141–153.

Angeler D.G., Sánchez-Carrillo S., García G. & Alvarez-Cobelas M. (2001) The influence of Procambarus clarkii (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. *Hydrobiologia* **464**, 89–98.

Baranov V., Lewandowski J., Romeijn P., Singer G. & Krause S. (2016) Effects of bioirrigation of non-biting midges (Diptera: Chironomidae) on lake sediment respiration. *Scientific Reports* **6**, 27329.

Barbaresi S., Santini G., Tricarico E. & Gherardi F. (2004a) Ranging behaviour of the invasive crayfish, Procambarus clarkii (Girard). *JOURNAL OF NATURAL HISTORY* **38**, 2821–2832.

Barbaresi S., Tricarico E. & Gherardi F. (2004b) Factors inducing the intense burrowing activity of the red-swamp crayfish, Procambarus clarkii, an invasive species. *Die Naturwissenschaften* **91**, 342–345.

Basnet K., Likens G.E., Scatena F.N. & Lugo A.E. (1992) Hurricane Hugo - damage to a tropical rain-forest in Puerto-Rico. *JOURNAL OF TROPICAL ECOLOGY* **8**, 47–55.

Bastviken D., Persson L., Odham G. & Tranvik L.J. (2004) Degradation of dissolved organic matter in oxic and anoxic lake water. *Limnology and Oceanography* **49**, 109–116.

Berrie A.D. (1992) The chalk-stream environment. *Hydrobiologia* **248**, 3–9.

Bilotta G.S. & Brazier R.E. (2008) Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research* **42**, 2849–2861.

Bovbjerg R. V (1970) Ecological isolation and competitive exclusion in two crayfish (Orconectes

virilis and Orconectes immunis). *Ecology* **51**, 225–236.

Boyd C. (1995) Bottom Soils, Sediment, and Pond Aquaculture. 113–148.

Brown L.E., Edwards F.K., Milner A.M., Woodward G. & Ledger M.E. (2011) Food web complexity and allometric scaling relationships in stream mesocosms: implications for experimentation. *Journal of Animal Ecology* **80**, 884–895.

Bubb D.H., Lucas M.C. & Thom T.J. (2002) Winter movements and activity of signal crayfish Pacifastacus leniusculus in an upland river, determined by radio telemetry. *Hydrobiologia* **483**, 111–119.

Callaway R.M. & Walker L.R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* **78**, 1958–1965.

Camargo J.A. & Alonso Á. (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International* **32**, 831–849.

Canal J., Laffaille P., Gilbert F., Lauzeral C. & Buisson L. (2015) Influence of temperature on surface sediment disturbance by freshwater fish: a microcosm experiment. *ANNALES DE LIMNOLOGIE-INTERNATIONAL JOURNAL OF LIMNOLOGY* **51**, 179–188.

Carpenter S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology* **77**, 677–680.

Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985) Cascading Trophic Interactions and Lake Productivity. *BioScience* **35**, 634–639.

Chucholl C. (2013) Feeding ecology and ecological impact of an alien “warm-water” omnivore in cold lakes. *Limnologica - Ecology and Management of Inland Waters* **43**, 219–229.

Cioni A. & Gherardi F. (2004) Agonism and interference competition in freshwater decapods. *Behaviour* **141**, 1297–1324.

Cottenie K., Nuytten N., Michels E. & De Meester L. (2001) Zooplankton community structure and environmental conditions in a set of interconnected ponds. *Hydrobiologia* **442**, 339–350.

Creed R.P. & Reed J.M. (2004) Ecosystem engineering by crayfish in a headwater stream community. *Journal of the North American Benthological Society* **23**, 224–236.

Creed Jnr R.P. (1994) Direct and indirect effects of crayfish grazing in a stream community. *Ecology* **75**, 2091–2103.

Croll S.L. & Watts S.A. (2004) The Effect of Temperature on Feed Consumption and Nutrient Absorption in Procambarus clarkii and Procambarus zonangulus. *Journal of the World Aquaculture Society* **35**, 478–488.

Crooks J.A. (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *OIKOS* **97**, 153–166.

Dalsgaard T. (ed), Nielsen L.P., Brotos V., Viaroli P., Underwood G., Nedwell D.B., Sundback K., Rysgaard S., Miles A., Bartoli M., Dong L., Thornton D.C.O., Ottosen L.D.M., G. C. & Risgaard-Petersen N. (2000) *Protocol handbook for NICE-Nitrogen Cycling in Estuaries: a project under the EU research programme: Marine Science and Technology (Mast III)*. National Environmental Research Institute, Department of Lake and Estuarine Ecology.

Davis K. & Huber R. (2007) Activity patterns, behavioural repertoires, and agonistic interactions of crayfish: A non-manipulative field study. *Behaviour* **144**, 229–247.

Davis W.R. (1993) The role of bioturbation in sediment resuspension and its interaction with physical shearing. *Journal of Experimental Marine Biology and Ecology* **171**, 187–200.

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.-I., Knowler D.J., Lévêque C., Naiman R.J., Prieur-Richard A.-H., Soto D., Stiassny M.L.J. & Sullivan C.A. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society* **81**, 163–82.

Ellis a., Jackson M.C., Jennings I., England J. & Phillips R. (2012) Present distribution and future spread of louisiana red swamp crayfish Procambarus clarkii (Crustacea, Decapoda, Astacida, Cambaridae) in Britain: Implications for conservation of native species and habitats. *Knowledge and Management of Aquatic Ecosystems* **406**, 5.

Elser J.J., Bracken M.E.S., Cleland E.E., Gruner D.S., Harpole W.S., Hillebrand H., Ngai J.T., Seabloom E.W., Shurin J.B. & Smith J.E. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**, 1135–1142.

Englund G. & Cooper S.D. (2003) Scale effects and extrapolation in ecological experiments. *Advances in Ecological Research* **33**, 161–213.

Espina S., Herrera F.D. & Bückle-Ramirez L.F. (1993) Preferred and avoided temperatures in the crawfish Procambarus clarkii (Decapoda, Cambaridae). *Journal of Thermal Biology* **18**, 35–39.

Evans-White M. a. & Lamberti G. a. (2005) Grazer species effects on epilithon nutrient composition. *Freshwater Biology* **50**, 1853–1863.

Feng J., Shen Z., Niu J. & Yang Z. (2008) The role of sediment resuspension duration in release of PAHs. *Chinese Science Bulletin* **53**, 2777–2782.

Feuchtmayr H. & Grey J. (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry* **17**, 2605–2610.

Fortino K. (2006) Effect of Season on the Impact of Ecosystem Engineers in the New River, NC. *Hydrobiologia* **559**, 463–466.

Geiger W., Alcorlo P., Baltanás A. & Montes C. (2005) Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Issues in Bioinvasion Science: EEI 2003: A Contribution to the Knowledge on Invasive Alien Species* **7**, 49–73.

Gherardi F. (2006) Crayfish invading Europe: the case study of Procambarus clarkii. *Marine and Freshwater Behaviour and Physiology* **39**, 175–191.

Gherardi F. & Acquistapace P. (2007) Invasive crayfish in Europe: The impact of Procambarus clarkii on the littoral community of a Mediterranean lake. *Freshwater Biology* **52**, 1249–1259.

Gherardi F., Aquiloni L., Dieguez-Uribeondo J. & Tricarico E. (2011) Managing invasive crayfish: is there a hope? *AQUATIC SCIENCES* **73**, 185–200.

Gherardi F., Barbaresi S. & Salvi G. (2000) Spatial and temporal patterns in the movement of Procambarus clarkii, an invasive crayfish. *Aquatic Sciences* **62**, 179–193.

Gherardi F. & Lazzara L. (2006) Effects of the density of an invasive crayfish (<I>Procambarus clarkii</I>) on pelagic and surface microalgae in a Mediterranean wetland. *Archiv für Hydrobiologie* **165**, 401–414.

Gil-Sánchez J.M. & Alba-Tercedor J. (2006) The Decline of the Endangered Populations of the Native Freshwater Crayfish (Austropotamobius pallipes) in Southern Spain: It is Possible to

Avoid Extinction? *Hydrobiologia* **559**, 113–122.

Greene C.H. (1983) Selective Predation in Freshwater Zooplankton Communities. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* **68**, 297–315.

Grey J. (2016) The Incredible Lightness of Being Methane-Fuelled: Stable Isotopes Reveal Alternative Energy Pathways in Aquatic Ecosystems and Beyond. *Frontiers in Ecology and Evolution* **4**, 8.

Grey J. & Jackson M.C. (2012) "leaves and eats shoots": Direct terrestrial feeding can supplement invasive red swamp crayfish in times of need. *PLoS ONE* **7**, e42575.

Grey J., Jones R.I. & Sleep D. (2000) Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. *Oecologia* **123**, 232–240.

Grey J., Kelly A. & Jones R.I. (2004) High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. *Limnology and Oceanography* **49**, 239–244.

Guan R.-Z. (1994) Burrowing behaviour of signal crayfish, Pacifastacus leniusculus (Dana), in the River Great Ouse, England. *Freshwater Forum* **4**, 144–168.

Guan R. & Roy Wiles P. (1996) Growth, density and biomass of crayfish, Pacifastacus leniusculus , in a British lowland river. *Aquatic Living Resources* **9**, 265–272.

Guildford S.J. & Hecky R.E. (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnology and Oceanography* **45**, 1213–1223.

Haas E.M., Kraak M.H.S., Koelmans A.A. & Admiraal W. (2005) The impact of sediment reworking by opportunistic chironomids on specialised mayflies. *Freshwater Biology* **50**, 770–780.

Hänfling B., Edwards F. & Gherardi F. (2011) Invasive alien Crustacea: Dispersal, establishment, impact and control. *BioControl* **56**, 573–595.

Harlioğlu M.M. (2009) A comparison of the growth and survival of two freshwater crayfish species, Astacus leptodactylus Eschscholtz and Pacifastacus leniusculus (Dana), under different temperature and density regimes. *Aquaculture International* **17**, 31–43.

Harvey G.L., Henshaw A.J., Moorhouse T.P., Clifford N.J., Holah H., Grey J. & Macdonald D.W. (2014) Invasive crayfish as drivers of fine sediment dynamics in rivers: Field and laboratory evidence. *Earth Surface Processes and Landforms* **39**, 259–271.

Harvey G.L., Moorhouse T.P., Clifford N.J., Henshaw a. J., Johnson M.F., Macdonald D.W., Reid I. & Rice S.P. (2011) Evaluating the role of invasive aquatic species as drivers of fine sediment-related river management problems: The case of the signal crayfish (Pacifastacus leniusculus). *Progress in Physical Geography* **35**, 517–533.

Hernandez L., Maeda-Martinez A.M., Ruiz-Campos G., Rodriguez-Almaraz G., Alonzo-Rojo F. & Sainz J.C. (2008) Geographic expansion of the invasive red crayfish Procambarus clarkii (Girard, 1852) (Crustacea : Decapoda) in Mexico. *BIOLOGICAL INVASIONS* **10**, 977–984.

Hobbs H.H. & Lodge D.M. (2010) Chapter 22 - Decapoda. In: *Ecology and Classification of North American Freshwater Invertebrates (Third Edition)*, Third Edit. (Eds J.H. Thorp & A.P. Covich), pp. 901–967. Academic Press, San Diego.

Hogger J.B. (1988) Ecology, Population Biology and Behaviour. In: *Freshwater Crayfish: Biology, Management and Exploitation*. (Eds D.M. Holdich & R.S. Lowery), pp. 114–144. Croom Helm Ltd.

Holdich D. (1999) The negative effects of established crayfish introductions. *CRAYFISH IN EUROPE AS ALIEN SPECIES: HOW TO MAKE THE BEST OF A BAD SITUATION* **11**, 31–47.

Holdich D.M. (2002) Distribution of Crayfish in Europe and Some Adjoining Countries. *Bulletin Français de la Pêche et de la Pisciculture*, 611–650.

Holdich D.M., James J., Jackson C. & Peay S. (2014) The North American signal crayfish, with particular reference to its success as an invasive species in Great Britain. *Ethology Ecology & Evolution* **26**, 232–262.

Holdich D.M. & Reeve I.D. (1991) Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and water quality. *Aquatic Conservation: Marine and Freshwater Ecosystems* **1**, 139–158.

Holdich D.M., Reynolds J.D., Souty-Grosset C. & Sibley P.J. (2009) A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* **394**–**395**, 11.

Holdich D.M., Rogers W.D. & Reynolds J.D. (1999) Native and alien crayfish in the British Isles. *Crayfish in Europe as Alien Species* **11**, 221–235.

Jackson M.C. & Grey J. (2013) Accelerating rates of freshwater invasions in the catchment of the River Thames. *Biological Invasions* **15**, 945–951.

Jackson M.C., Jones T., Milligan M., Sheath D., Taylor J., Ellis A., England J. & Grey J. (2014) Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshwater Biology* **59**, 1123–1135.

James J., Thomas J.R., Ellis A., Young K.A., England J. & Cable J. (2016) Over-invasion in a freshwater ecosystem: newly introduced virile crayfish (Orconectes virilis) outcompete established invasive signal crayfish (Pacifastacus leniusculus). *Marine and Freshwater Behaviour and Physiology* **49**, 9–18.

Johnson M.F., Rice S.P. & Reid I. (2014) The activity of signal crayfish (Pacifastacus leniusculus) in relation to thermal and hydraulic dynamics of an alluvial stream, UK. *Hydrobiologia* **724**, 41–54.

Johnson M.F., Rice S.P. & Reid I. (2010) Topographic disturbance of subaqueous gravel substrates by signal crayfish (Pacifastacus leniusculus). *Geomorphology* **123**, 269–278.

Jones C.G., Lawron J.H. & Shachak M. (1997) Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* **78**, 1946–1957.

Jones C.G., Lawton J.H. & Shachak M. (1994) Organisms as Ecosystem Engineers. *Oikos* **69**, 373–386.

Jones R.I. & Grey J. (2011) Biogenic methane in freshwater food webs. *Freshwater Biology* **56**, 213–229.

Kajan R. & Frenzel P. (1999) The effect of chironomid larvae on production, oxidation and fluxes of methane in a flooded rice soil. *FEMS Microbiology Ecology* **28**, 121–129.

Kankaala P., Taipale S., Li L. & Jones R.I. (2010) Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquatic Ecology* **44**, 781–795.

Kerkut G.A. & Taylor B.J.R. (1958) The Effect of Temperature Changes On the Activity of Poikilotherms. *Behaviour* **13**, 259–279.

Kouba A., Petrusek A. & Kozák P. (2014) Continental-wide distribution of crayfish species in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems*, 5.

Larson E.R. & Olden J.D. (2011) The State of Crayfish in the Pacific Northwest. *Fisheries* **36**, 60–73.

Ledger M.E., Harris R.M.L., Armitage P.D. & Milner A.M. (2009) Realism of model ecosystems: an evaluation of physicochemistry and macroinvertebrate assemblages in artificial streams. *Hydrobiologia* **617**, 91–99.

Lennon J.T., Faiia A.M., Feng X. & Cottingham K.L. (2006) Relative importance of CO2 recycling and CH4 pathways in lake food webs along a dissolved organic carbon gradient. *Limnology and Oceanography* **51**, 1602–1613.

Lodge D.M., Deines A., Gherardi F., Yeo D.C.J., Arcella T., Baldridge A.K., Barnes M.A., Chadderton W.L., Feder J.L., Gantz C.A., Howard G.W., Jerde C.L., Peters B.W., Peters J.A., Sargent L.W., Turner C.R., Wittmann M.E. & Zeng Y. (2012) Global Introductions of Crayfishes: Evaluating the Impact of Species Invasions on Ecosystem Services. In: *ANNUAL REVIEW OF ECOLOGY, EVOLUTION, AND SYSTEMATICS, VOL 43*. Annual Review of Ecology Evolution and Systematics, (Ed. Futuyma, DJ), p. 449+. ANNUAL REVIEWS, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0897 USA.

Lodge D.M., Kershner M.W., Aloi J.E. & Covich a. P. (1994) Effects of an omnivorous crayfish (Orconectes rusticus) on a freshwater littoral food web. *Ecology* **75**, 1265–1281.

Maberly S.C., King L., Dent M.M., Jones R.I. & Gibson C.E. (2002) Nutrient limitation of phytoplankton and periphyton growth in upland lakes. *Freshwater Biology* **47**, 2136–2152.

Mermillod-Blondin F., Gaudet J.-P., Gerino M., Desrosiers G., Jose J. & Châtelliers M.C. des (2004) Relative influence of bioturbation and predation on organic matter processing in river sediments: a microcosm experiment. *Freshwater Biology* **49**, 895–912.

Meysman F.J.R., Middelburg J.J. & Heip C.H.R. (2006) Bioturbation: a fresh look at Darwin's last idea. *Trends in Ecology and Evolution* **21**, 688–695.

Momot W., Gowing H. & Jones P. (1978) The dynamics of crayfish and their role in ecosystems. *American Midland Naturalist* **99**, 10–35.

Nogaro G., Mermillod-Blondin F., Montuelle B., Boisson J.C. & Gibert J. (2008) Chironomid larvae stimulate biogeochemical and microbial processes in a riverbed covered with fine sediment. *Aquatic Sciences* **70**, 156–168.

Nystrom P., Bronmak C. & Graneli W. (1999) Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* **85**, 545–553.

Nystrom P., Bronmark C. & Graneli W. (1996) Patterns in benthic food webs: a role for

omnivorous crayfish? *Freshwater Biology* **36**, 631–646.

Paerl H.W. & Otten T.G. (2013) Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology* **65**, 995–1010.

Parkyn S.M., Rabeni C.F. & Collier K.J. (1997) : Parastacidae) on in-stream processes and benthic faunas: A density manipulation experiment. *New Zealand Journal of Marine and Freshwater Research* **31**, 685–692.

Peterson C.J., Carson W.P., McCarthy B.C. & Pickett S.T.A. (1990) Microsite Variation and Soil Dynamics within Newly Created Treefall Pits and Mounds. *Oikos* **58**, 39–46.

Poff N.L. & Ward J. V (1989) Implications of Streamflow Variability and Predictability for Lotic Community Structure: A Regional Analysis of Streamflow Patterns. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 1805–1818.

Power M.E., Stout R.J., Cushing C.E., Harper P.P., Hauer F.R., Matthews W.J., Moyle P.B., Statzner B. & Badgen I.R.W. De (1988) Biotic and Abiotic Controls in River and Stream Communities. *Journal of the North American Benthological Society* **7**, 456–479.

Puttock A., Graham H.A., Cunliffe A.M., Elliott M. & Brazier R.E. (2017) Eurasian beaver activity increases water storage, attenuates flow and mitigates diffuse pollution from intensively-managed grasslands. *Science of the Total Environment* **576**, 430–443.

Ravinet M., Syväranta J., Jones R.I. & Grey J. (2010) A trophic pathway from biogenic methane supports fish biomass in a temperate lake ecosystem. *Oikos* **119**, 409–416.

Renai B. & Gherardi F. (2004) Predatory Efficiency of Crayfish: Comparison Between Indigenous and Non-Indigenous Species. *Biological Invasions* **6**, 89–99.

Rice S.P., Johnson M.F., Mathers K., Reeds J. & Extence C. (2016) The importance of biotic entrainment for base flow fluvial sediment transport. *JOURNAL OF GEOPHYSICAL RESEARCH-EARTH SURFACE* **121**, 890–906.

Ritvo G., Kochba M. & Avnimelech Y. (2004) The effects of common carp bioturbation on fishpond bottom soil. *Aquaculture* **242**, 345–356.

Rodríguez C.F., Bécares E. & Fernández-Aláez M. (2003) Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (Procambarus clarkii). *Hydrobiologia* **506**–**509**, 421–426.

Rodríguez G. & Suárez H. (2001) Anthropogenic dispersal of decapod crustaceans in aquatic environments. *Interciencia* **26**, 282–288.

Ruokonen T.J., Karjalainen J., Kiljunen M., Pursiainen M. & Hämäläinen H. (2012) Do introduced crayfish affect benthic fish in stony littoral habitats of large boreal lakes? *Biological Invasions* **14**, 813–825.

Sanders I.A., Heppell C.M., Cotton J.A., Wharton G., Hildrew A.G., Flowers E.J. & Trimmer M. (2007) Emission of methane from chalk streams has potential implications for agricultural practices. *FRESHWATER BIOLOGY* **52**, 1176–1186.

Sanseverino A.M., Bastviken D., Sundh I., Pickova J. & Enrich-Prast A. (2012) Methane Carbon Supports Aquatic Food Webs to the Fish Level. *PLOS ONE* **7**, 1–8.

Savolainen R., Ruohonen K. & Railo E. (2004) Effect of stocking density on growth, survival and cheliped injuries of stage 2 juvenile signal crayfish Pasifastacus leniusculus Dana. *Aquaculture* **231**, 237–248.

Schaus M.H. & Vanni M.J. (2000) Effects of gizzard shad on phytoplankton and nutrient dynamics: Role of sediment feeding and fish size. *ECOLOGY* **81**, 1701–1719.

Schindler D.W. (1977) Evolution of Phosphorus Limitation in Lakes. *Science* **195**, 260–262.

Schindler D.W. (1998) Replication versus Realism: The Need for Ecosystem-Scale Experiments. *Ecosystems* **1**, 323–334.

Seals C., Eversole A.G., Tomasso J.R. & Petrosky B.R. (1997) Effects of Temperature on Feeding Activity of the White River Crayfish Procambarus acutus acutus1. *Journal of the World Aquaculture Society* **28**, 133–141.

Seitz R.D., Dauer D.M., Llansó R.J. & Long W.C. (2009) Broad-scale effects of hypoxia on benthic community structure in Chesapeake Bay, USA. *Journal of Experimental Marine Biology and Ecology* **381**, S4–S12.

Shelley F., Grey J. & Trimmer M. (2014) Widespread methanotrophic primary production in lowland chalk rivers. *Proceedings of the Royal Society of London B: Biological Sciences* **281**.

Smith J.M. & Mather M.E. (2013) Beaver dams maintain fish biodiversity by increasing habitat heterogeneity throughout a low-gradient stream network. *Freshwater Biology* **58**, 1523–1538.

Smith V.H., Tilman G.D. & Nekola J.C. (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* **100**, 179–196.

Strayer D.L. (2010) Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* **55**, 152–174.

Taipale S., Kankaala P., Hämäläinen H. & Jones R.I. (2009) Seasonal shifts in the diet of lake zooplankton revealed by phospholipid fatty acid analysis. *Freshwater Biology* **54**, 90–104.

Taipale S., Kankaala P. & Jones R.I. (2007) Contributions of Different Organic Carbon Sources to Daphnia in the Pelagic Foodweb of a Small Polyhumic Lake: Results from Mesocosm DI13C-Additions. *Ecosystems* **10**, 757–772.

Tisdall J.M. & Oades J.M. (1982) Organic matter and water-stable aggregates in soils. *Journal of Soil Science* **33**, 141–163.

Trimmer M., Hildrew A.G., Jackson M.C., Pretty J.L. & Grey J. (2009) Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. *Limnology and Oceanography* **54**, 1541–1547.

Usio N. & Townsend C.R. (2004) Roles of crayfish: Consequences of predation and bioturbation for stream invertebrates. *Ecology* **85**, 807–822.

Vuorio K., Meili M. & Sarvala J. (2006) Taxon-specific variation in the stable isotopic signatures (δ13C and δ15N) of lake phytoplankton. *Freshwater Biology* **51**, 807–822.

Walling D.E. & Amos C.M. (1999) Source, storage and mobilisation of fine sediment in a chalk stream system. *HYDROLOGICAL PROCESSES* **13**, 323–340.

Westbrook C.J., Cooper D.J. & Butler D.R. (2013) Beaver Hydrology and Geomorphology. In: *Treatise on Geomorphology*. pp. 293–306.

Willis-Jones E., Jackson M.C. & Grey J. (2016) Environmental Drivers for Population Success: Population Biology, Population and Community Dynamics. In: *Biology and Ecology of Crayfish*. (Eds M. Longshaw & P. Stebbing), pp. 251–286. CRC Press.

Wood P.J. (1997) Biological Effects of Fine Sediment in the Lotic Environment. *Environmental Management* **21**, 203–217.

Wright J.P., Jones C.G. & Flecker A.S. (2002) An ecosystem engineer, the beaver, increases species richness at the landscape scale. *Oecologia* **132**, 96–101.

Yamamoto Y. (2010) Contribution of bioturbation by the red swamp crayfish Procambarus clarkii to the recruitment of bloom-forming cyanobacteria from sediment. *Journal of Limnology* **69**, 102.

Yvon-Durocher G., Jones J.I., Trimmer M., Woodward G. & Montoya J.M. (2010) Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **365**, 2117–2126.

Zhang Y., Richardson J.S. & Negishi J.N. (2004) Detritus processing, ecosystem engineering and benthic diversity: A test of predator-omnivore interference. *Journal of Animal Ecology* **73**, 756–766.